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OBSERVATIONS ON CACAO POD PRODUCTION IN RELATION TO THE INCIDENCE OF BLACK-POD DISEASE, CAUSED BY *PHYTOPHTHORA PALMIVORA*

By C. A. THOROLD

Department of Agricultural Research, Nigeria

THE rotting of cacao fruits caused by *Phytophthora palmivora* is generally known as black-pod disease. This disease is responsible for annual losses in Nigeria estimated as equivalent to 15,000 tons of dried beans (Anon. (1)).

Thorold has shown (8, 9) that certain treatments can reduce losses from black-pod disease by altering the proportion of diseased to total pods. It is important to know whether black-pod percentages and the total numbers of pods are associated or independent, because, if these two variables are directly associated, measures or factors which influence black-pod percentages may also be reflected in total pod numbers. The object of this paper is to consider certain factors affecting black-pod percentages and to examine their relationship to total pod numbers.

In experiments conducted to find methods of controlling this disease, it has been convenient to assess the results of treatments on a basis of the numbers of black pods and of healthy pods present on treated and untreated trees during the course of the cacao season, which conventionally runs from the 1st of April to the 31st of March.

It is sometimes convenient to summarize incidence in terms of black pods expressed as a percentage of the total number of pods (healthy plus diseased). This procedure is based on an assumption that the total number of pods produced is not affected by any of the treatments imposed on the cacao tree populations which are being compared. Sometimes there have been reasons to doubt the validity of this assumption. Reynaud (6) has described spraying experiments in the Ivory Coast, in which a reduction in number of affected pods appeared to be accompanied by a reduction in the total number of pods in the sprayed plots, as compared with the untreated plots. In those trials, one application of 4 per cent. Bordeaux mixture was given in 1950, followed by one application of 5 per cent. Bordeaux mixture in 1951. Reynaud (*loc. cit.*) did not observe leaf scorching at these concentrations, but he suggested that the apparent reduction in total number of pods might be due to an adverse effect of the fungicide on pollination or fruit setting. The writer did not find an effect on pod production in the course of two seasons when 1 per cent. carbide-Bordeaux mixture was applied with intervals of only one to four weeks between applications in small-scale, replicated plot experiments (8). In field trials, however, with a similar concentration of fungicide (9), the mean total number of pods harvested was smaller for sprayed trees (13·4) than for unsprayed (14·6). Attention should also be drawn to reports of similar effects in cacao elsewhere by Constain (2), Naundorf (4) and Polania Trujillo (5).

EXPERIMENTS AND RESULTS.

Frequency of black-pod removal.

The writer has shown (9) that inspection of the trees every other day for removal of black pods in the earliest symptom stage reduces the number of black pods as compared with a less frequent removal. Such indications were found when two adjacent areas of cacao were compared, in one of which the trees were inspected every other day (frequent removal) and in the other at monthly intervals (infrequent removal) during two consecutive seasons. The results are summarized in Table I which shows that black-pod percentages were smaller when diseased pods were removed frequently.

TABLE I.

Mean numbers of black pods, as percentage of total pods (%B.P.), and of total pods (T.P.) per tree, in two areas of cacao in which black pods were removed frequently or infrequently.

Treatment	1950/51			1951/52		Mean of two seasons	
	No. of trees	B.P. %	T.P.	B.P. %	T.P.	B.P. %	T.P.
Infrequent removal ..	303	83·8	13·1	96·7	12·6	90·1	12·9
Frequent removal	579	48·6	13·3	73·1	11·0	59·7	12·2

Effect of different spacings.

The two areas considered above were not uniform, because in each area there were two blocks in one of which the trees were widely spaced (15 ft. × 15 ft.) and in the other closely spaced (8 ft. × 8 ft.). The combined results for the two seasons are given in Table II, from which it is seen that, at both spacings, the black-pod per-

TABLE II.

Mean numbers of black pods, as percentage of total pods (%B.P.) per season, and of total pods (T.P.) per tree per season, for wide and close spacings, in each of which black pods were removed frequently or infrequently.

Treatment	Spacing					
	8 ft. × 8 ft.			15 ft. × 15 ft.		
	No. of trees	B.P. %	T.P.	No. of trees	B.P. %	T.P.
Infrequent removal ..	178	86·8	9·9	125	92·9	17·1
Frequent removal ..	383	57·0	11·5	196	64·1	13·4

centages were less when diseased pods were removed frequently. The total number of pods per tree was greater at the wider spacing, which is in accordance with observations discussed by Russell (7). At both spacings the range in total numbers of pods

was considerable, namely, 0 to 175 pods per tree at 15 ft. × 15 ft. and 0 to 119 pods per tree at 8 ft. × 8 ft.

The data summarized in Tables I and II give only the overall averages for black-pod percentages and total pod numbers. For the purpose of this investigation it was desirable to ascertain average black-pod percentages for varying total numbers of pods. Such relationships were conveniently determined by covariance analysis and the relevant regression coefficients are given in Table III below.

Relationship between black-pod incidence and total number of pods per tree.

In the Ivory Coast, Reynaud (6) found that the percentage of diseased pods was directly proportional to the total number of pods up to about 25 pods per tree, but that for larger individual tree yields an inverse relationship appeared to develop.

To compare the observations in Nigeria with those made in the Ivory Coast, the proportion of black pods to the total number of pods per tree was considered. Regression coefficients were determined for black-pod percentages (y) by the angular transformation of Fisher and Yates (3) upon the total number of pods (x). This procedure was applied to data from trees which produced at least one pod in each of two consecutive seasons.

TABLE III.

Values of the regression coefficient (b_{xy}) for black-pod percentages (transformed) on total pods, for trees at two spacings in two seasons, in which black pods were removed frequently or infrequently.

Treatment	1950/51				1951/52			
	Spacing				Spacing			
	8 ft. × 8 ft.		15 ft. × 15 ft.		8 ft. × 8 ft.		15 ft. × 15 ft.	
	No. of trees	b_{xy}	No. of trees	b_{xy}	No. of trees	b_{xy}	No. of trees	b_{xy}
Infrequent removal	116	+0·30	92	+0·12	116	+0·13	92	+0·11
Frequent removal	276	+0·40*	123	+0·21	276	+0·68*	123	+0·35*

* Significant at the 1 per cent. level.

The regression coefficients given in Table III show that, in all cases but one, the values differed significantly from zero when black pods were removed frequently, the one exception ($b_{xy} = +0·21$) only just missing significance at the 5 per cent. level. By contrast, black-pod percentages appeared to be independent of total pod numbers when black pods were removed infrequently. In this latter case it seems probable that the relatively unrestricted occurrence of the disease tended to produce a uniform disease incidence and thus to make black-pod percentages independent of total pod numbers.

Under other conditions, these variables may be directly proportional, as was found by Reynaud (6) in the Ivory Coast, though he did not indicate either the length of the intervals between the four harvestings made by him nor the frequency of black-pod removal. There was also no evidence of an inverse relationship with relatively large total pod numbers in Nigeria. However, within the population of

607 trees so far considered (Table III), there were relatively few (22 per cent.) yielding more than 25 pods, which Reynaud found to be about the critical point for the change from a positive to a negative association between black-pod percentages and total pod numbers.

To obtain information on this point, records were examined for a relatively high-yielding population of 118 trees in the Cameroons, which were recorded individually in 1954 when ripe pods were harvested and black pods removed at intervals of two weeks. The total crop per tree averaged 44 pods, and black-pod incidence was relatively severe (66 per cent.). These observations are summarized in Table IV, in which individual tree records are grouped according to total pod numbers. In these relatively high-yielding trees the mean black-pod percentage was greatest

TABLE IV.

Classification of 118 trees observed in 1954 at Kumba, Cameroons.

Yield class (Total pods)	Frequency (No. of trees)	Mean % B.P.
1–19	25	57·9
20–39	35	65·9
40–59	32	65·3
60 and over	26	68·5

(68·5 per cent.) when yields were large (60 or more per tree), and least (57·9) for the lowest yield class (1 to 19 pods per tree). This information accords with the writer's observation (9) that, with relatively few pods per tree, the number of black pods tends to be less than expectation, whereas, with relatively large numbers of pods per tree, the number of black pods tends to be greater than expectation; it is contrary, however, to Reynaud's finding of a decline in black-pod percentages with relatively large yields.

Association of black-pod incidence early in the season with total pod number.

Observations made on a large number of trees at Owena showed that there was a sequence of black-pod incidence during the cacao season, the disease reaching a peak between August and October. The maximum number of healthy pods was harvested between October and December. Table V shows these trends for the period 1950–52.

TABLE V.

Numbers of black pods (B.P.) and of healthy pods (H.P.) each month at Owena, as percentage of the yearly totals of black pods and healthy pods respectively, in two seasons.

	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
1950/51												
%B.P.	0·0	0·0	0·6	13·0	7·2	20·2	41·1	12·2	1·0	3·4	1·3	0·0
%H.P.	1·4	4·2	1·6	0·3	0·5	3·9	34·7	19·2	20·5	13·0	0·7	0·0
1951/52												
%B.P.	0·0	0·0	1·8	16·0	25·0	20·1	22·0	13·6	1·3	0·2	0·0	0·0
%H.P.	0·1	5·2	4·0	6·6	3·4	11·3	16·0	16·3	27·1	9·2	0·8	0·0

Practically no black pods occurred in March, April or May. It seemed possible that a relatively early occurrence of black pods in the cacao season, starting in April, might affect total pod production. To examine this possibility, a measure of earliness was devised, on the suggestion of Mr. G. M. Jolly, in which the numbers of black pods per tree per month were represented as: May, W_3; June, W_2; July, W_1 and August to April, W_0. Earliness (E) was determined from the formula

$$E = \frac{3W_3+2W_2+1W_1+0W_0}{W_3+W_2+W_1+W_0}.$$

E was calculated for those individual trees which produced a total of one or more pods in each of two consecutive seasons (1950/51 and 1951/52). The values of the correlation coefficients between earliness (E) and total pods are given in Table VI, which shows that there were small but significant positive correlations when black pods were removed frequently, but none when they were removed infrequently.

TABLE VI.

Correlation coefficients (r) between "earliness" of black-pod occurrence and total number of pods, for trees at two spacings, in two seasons in which black pods were removed frequently or infrequently.

Treatment	1950/51				1951/52			
	Spacing				Spacing			
	8 ft. × 8 ft.		15 ft. × 15 ft.		8 ft. × 8 ft.		15 ft. × 15 ft.	
	No. of trees	r	No. of trees	r	No. of trees	r	No. of trees	r
Infrequent removal	116	−0·079	92	−0·003	116	−0·094	92	+0·170
Frequent removal	276	+0·231†	123	+0·182*	276	+0·193†	123	+0·198*

* Significant at the 5 per cent. level.
† Significant at the 1 per cent. level.

DISCUSSION.

Consideration has been given to the effects of two frequencies of black-pod removal on black-pod percentages and on the association of these percentages with total pod numbers. It has been shown that, under conditions of frequent black-pod removal, an increase in the total number of pods is accompanied by an increase in black-pod percentages. When black pods are removed frequently, there is evidence that their occurrence early in the season may be associated with greater total numbers of pods.

In the data considered here comparisons have only been made between the frequent removal of diseased pods every other day and their infrequent removal once a month. No information is presented as to the possible association between black-pod percentages and total pod numbers in relation to intermediate frequencies of removal in Nigeria. Wickens (11) found that at two sites in the Gold Coast there were significant increases in the numbers of pods per tree associated with

decreasing intervals between pickings. There were four such intervals under observation, 3, 6, 12 and 24 days. The numbers of pods per tree for one site (Tafo) ranged from 7·90 (3-day frequency) to 3·64 (24-day frequency), the two other values being intermediate. Wharton (10) also reported that, in a spraying and harvesting trial in the Gold Coast, there was a greater number of total pods per tree in plots harvested weekly (38·54) than in those harvested once a month (28·00).

Although it is not possible to define the limits of " frequent " as opposed to " infrequent " removal in Nigeria, it seems likely that the relationship between total numbers of pods and black-pod percentages, which applies under circumstances of frequent removal, is gradually modified towards independence when removal becomes less frequent. This affords a possible explanation for those circumstances, in which control measures, leading to small black-pod percentages, may result in the production of smaller total numbers of pods than are found on untreated trees carrying higher percentages of diseased pods.

SUMMARY.

1. There is a tendency for black-pod percentages to be positively associated with the total numbers of pods in certain circumstances.

2. When black pods are removed frequently, their occurrence early in the season may be associated with greater total numbers of pods.

3. These associations between black-pod percentages and total numbers of pods are considered in relation to control measures which aim at reducing black-pod percentages.

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OBSERVATIONS ON THE ORIGIN AND DEVELOPMENT OF SPHAEROBLASTS IN THE APPLE

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In 1891 Krick reviewed the extensive literature dealing with the small woody inclusions that occur in the bark of many trees and protrude more or less conspicuously from their trunks. They were described under such names as nodules ligneux, loupes, Knollenmasern, Maserknollen, Holzkugeln, Holzknollen, Rindenknollen, Kugeltriebe or Sphäroblaste, and detailed and accurate descriptions of their origin and structure exist. Though all these structures bear considerable superficial resemblance, their method of origin and subsequent development seem to vary in different species.

Sphaeroblasts, according to present-day usage, are defined† as "nodules of wood, separated from the central woody cylinder, and surrounded by bark". Interest in them revived in 1937 with Stoutemyer's observation that truly adventitious shoots with "juvenile" characters could develop from the sphaeroblasts of apples and pears. The significance of this observation lies in the possibility of restoring the "juvenile" phase in the "mature" plant. In this phase shoots show an increased capacity to form roots, and sphaeroblasts may thus provide an aid to the vegetative propagation of species and varieties otherwise difficult to root, as was pointed out by Wellensiek (1952) and by Hatcher and Garner (1955). These authors, following a technique first used by Dermen (1948), stimulated the production of adventitious buds by a complete disbudding treatment, and were able to induce sphaeroblast formation on an experimental scale. As a result of the investigations at East Malling, Crab C apple rootstocks with artificially induced sphaeroblasts in different stages of development were available. They provided the material for a detailed study of the exact location of the sphaeroblast primordia, their histological structure, and the way in which shoots arose from them. These studies are described in the present paper.

Material.

Stems of Crab C with and without sphaeroblasts were examined. Those with sphaeroblasts had been produced by cutting back two-year-old lined out rootstocks to three inches above ground level in March 1954 and by allowing one vigorous shoot to develop. This was cut to three feet above ground level in March 1955 and all visible buds were removed. Subsequent shoots from dormant buds were rubbed out as soon as they arose, and sphaeroblasts began to form at the end of May. The first sphaeroblastic shoots grew out two months later. Untreated Crab C shoots

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† "Webster's New International Dictionary of the English Language." Second edition, 1950.

of similar age (approximately 1½ years) and diameter were used as controls. These shoots were from young trees that had not been pruned back.

Naturally occurring sphaeroblasts of apple, pear, holly, fig and laburnum were also examined.

Method.

In June and July 1955, some twenty Crab C sphaeroblasts in different stages of development (including shoot formation) were examined microscopically by cutting serial transverse and longitudinal sections of the fresh material on a sledge microtome. Since sphaeroblasts visible to the naked eye proved to be too far advanced for determination of the exact method of their initiation, some further sections were cut of the bark of disbudded stems which had not yet produced sphaeroblasts. Certain histological changes which appeared to precede sphaeroblast formation were observed in this material, and a comparison was made of the bark structure of six disbudded and six control Crab C stems. Internodal transverse sections (not in the vicinity of a developing sphaeroblast) were cut, and measurements were made of the radial width of the various bark tissues, i.e., collenchyma, cortical parenchyma and phloem, and of the mean cell size in the first two tissues. Sections were stained in safranin and aniline blue by the following method, which has been found successful at East Malling for showing the details of bark structure.

The gradually dehydrated sections were stained in a 1 per cent. solution of safranin in absolute alcohol for 10–15 minutes, after which excess dye was removed by two rapid washes in absolute alcohol. Sections were counterstained in a 1 per cent. solution of aniline blue in cellosolve (ethylene glycol monoethyl ether) for 1–3 minutes and then rinsed twice in cellosolve before final clearing in clove oil. After removal of excess clove oil by rinsing in absolute alcohol, the sections were transferred to xylol and mounted in Canada balsam.

Results.

Changes induced in the bark structure by the disbudding treatment.

Typical transverse sections of the bark of normal and disbudded stems are shown in Fig. 1, a and b. The most striking difference between them was that the disbudded stems had made no growth from the cambium during the spring and summer of 1955 when they were not carrying any shoots. In spite of this, the bark separated without difficulty from the wood of the disbudded stems and actually appeared to be slightly thicker than that of control stems. Microscopical examination showed that, although there had been no growth from the vascular cambium, a widespread resumption of meristematic activity had occurred in many cells of the cortex and in the outer part of the phloem laid down in 1954. The measurements shown in Table I support these observations.

Resumption of meristematic activity occurred not only in the cortex (collenchyma and cortical parenchyma) which doubled its radial width, but spread also to the phloem laid down in 1954 which (see Table I) extended its radial width by about one quarter. Some of the increased bark tissue of disbudded stems was due to cell expansion rather than cell division. This is shown by the measurements given in Table II.

TABLE I.

Radial width of bark, collenchyma, cortical parenchyma and phloem in disbudded and normal stems of Crab C (mean of 6 stems).

Tissue	Normal stems μ	Disbudded stems μ	Level of significance of difference
Collenchyma	66	116	0·1%
Cortical parenchyma	160	320	1%
Total phloem, 1954 and 1955	696	640	Not significant
Phloem, 1954	506	640	5%
Phloem, 1955	190	0	—
Total bark	*922*	*1,076*	*1%*

TABLE II.

Mean area of collenchyma and cortical parenchyma cells in transverse sections of disbudded and normal stems of Crab C (mean of ten measurements from each of three stems).

Tissue	Normal stems μ^2	Disbudded stems μ^2	Level of significance of difference
Collenchyma	574	798	1%
Cortical parenchyma	1,008	1,329	1%

Origin and subsequent development of sphaeroblasts in disbudded stems.

Stages of development in the formation of artificially induced sphaeroblasts are shown in diagrammatic form in Fig. 2. Sphaeroblast initiation is preceded by a marked local increase in the uncoordinated meristematic activity of the bark tissue following disbudding. The bark in such places may attain twice its normal thickness, and in this rapidly dividing tissue a meristematic centre with densely staining thin-walled cells, not unlike an apical meristem, is formed (Fig. 3, a and b). In all the specimens examined, the first meristematic centre developed in the cortex, as shown in Fig. 2(i), but its initiation was rapidly followed by the formation of other centres close to it, and some of these occurred in the outer phloem. For purposes of this description all regions within the primary phloem fibre ring are regarded as phloem, and all those outside it as cortex. Fusion of the different meristematic centres and of their derivative tissues occurs, and a definite rounded body with several growth centres is formed (Fig. 3c). This body may be regarded as the pro-sphaeroblast. It is as yet purely meristematic, unlignified and undifferentiated, with none of the xylem elements characteristic of mature sphaeroblasts. Occasionally it may include within its confines existing phloem fibre groups. Further development of the pro-sphaeroblast follows one of two courses. Either a proper apical meristem is developed in the sphaeroblast, which then goes through the developmental stages shown in Figs. 2 (iib to v) and 3d and finally produces one or

PLATE I.

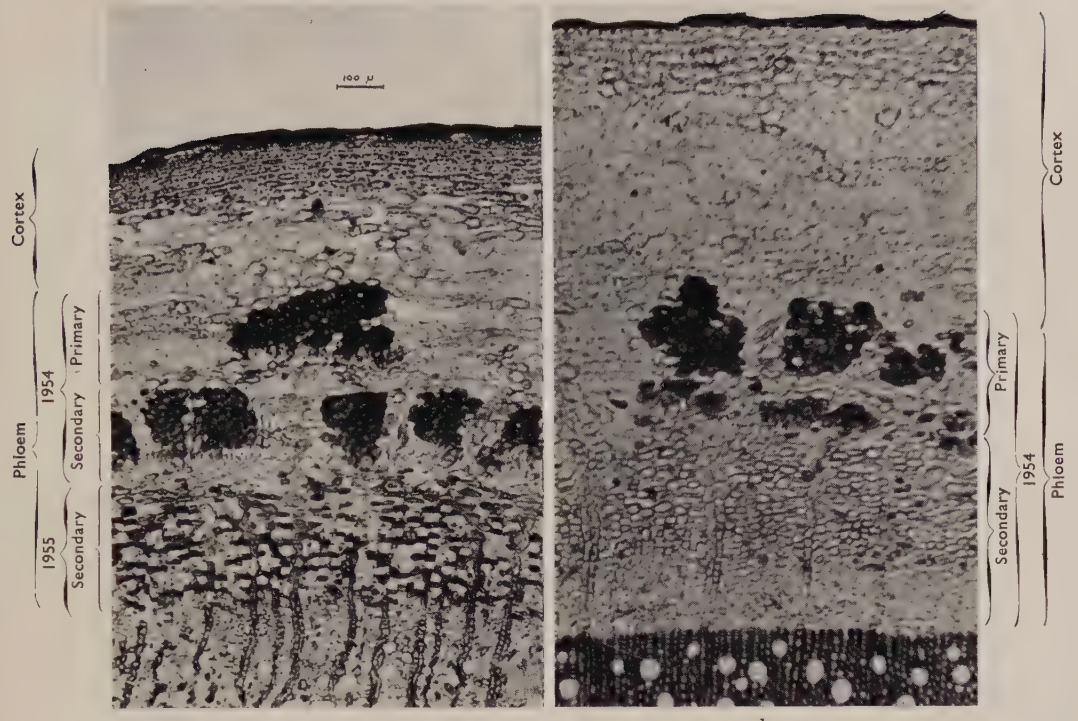

Fig. 1.

Transverse sections of the bark of stems ($1\frac{1}{2}$ years old) of Crab C apple ; (a) control, (b) disbudded.

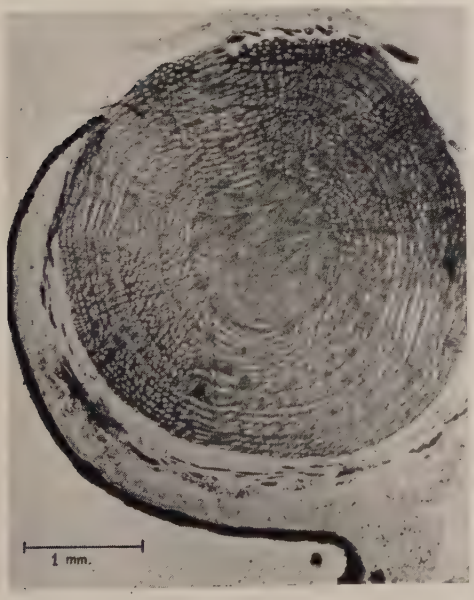

Fig. 4.

Transverse section of a four-year-old sphaeroblast that occurred naturally in the stem of a pear tree.

Fig. 5.

Radial longitudinal section showing the connection between a sphaeroblast shoot and the disbudded Crab C stem from which it arose.

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PLATE II.

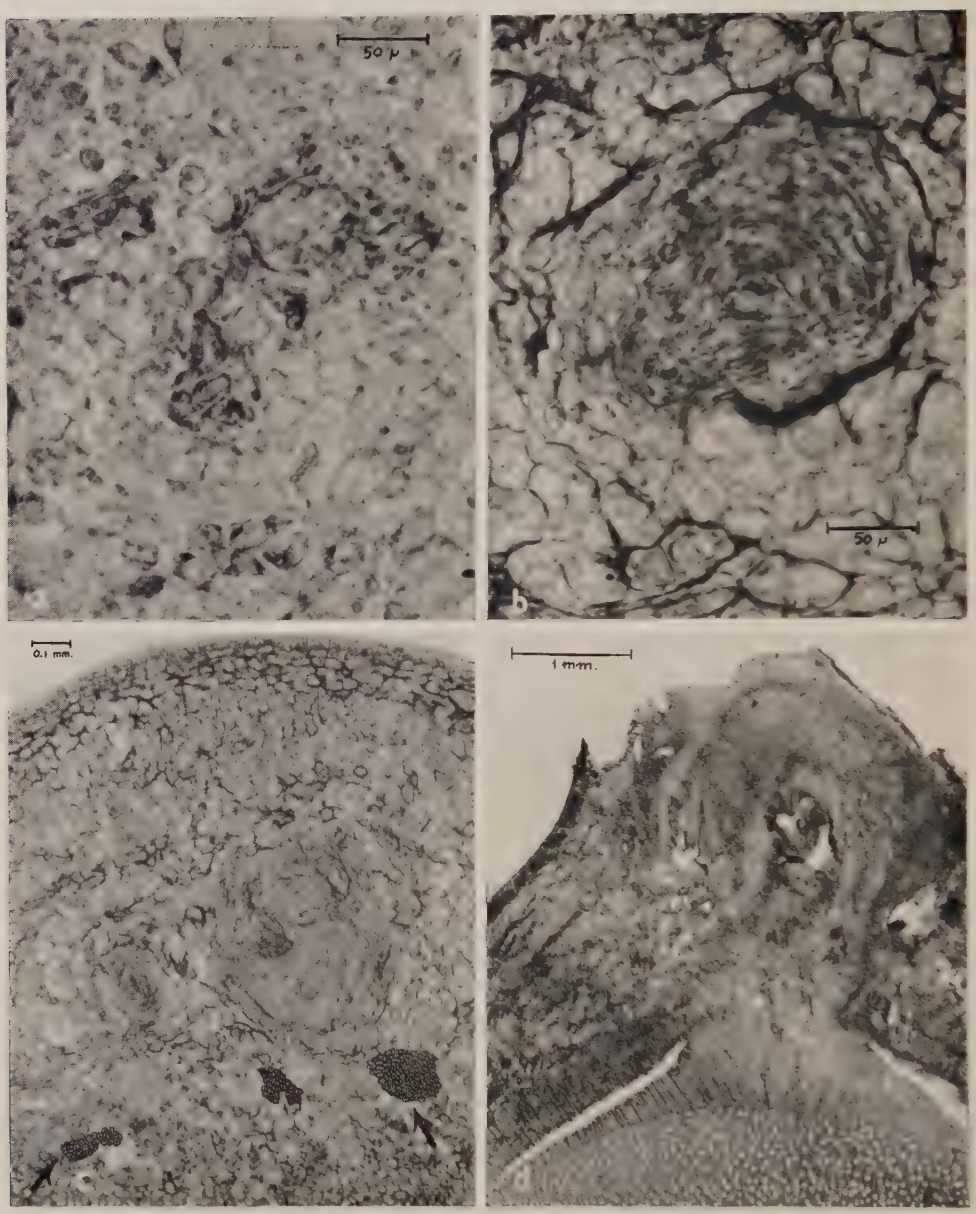

FIG. 3.

Transverse sections of developing sphaeroblasts in disbudded Crab C stems.

a. Sphaeroblast initial.
b. Developing sphaeroblast.
c. Sphaeroblast with several centres, developing in the cortex. Groups of primary phloem fibres are indicated by arrows.
d. Sprouting sphaeroblast with basal callus pad.

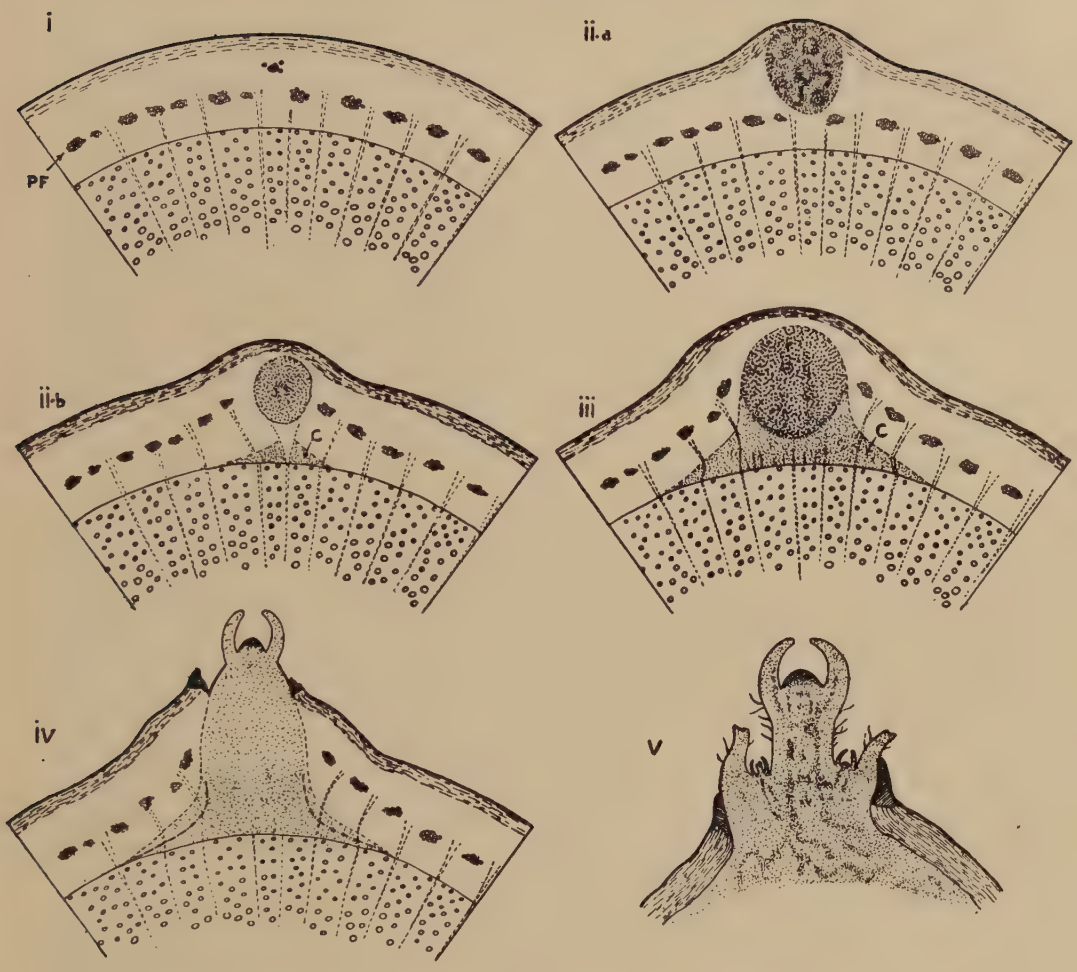

Fig. 2.

Diagrams showing origin and development of sphaeroblasts in disbudded Crab C stems.
i. Sphaeroblast initials. PF, primary phloem fibres.
iia. Early stage in the formation of a lignified sphaeroblast.
iib–v. Stages in the formation of a sphaeroblastic shoot ; C, callus-like connecting tissue.

more shoots (Fig. 5), or no apical meristem is differentiated, and a typical woody sphaeroblast is formed as shown in Figs. 2 (iia) and 4.

Those sphaeroblasts which immediately proceed to form shoots are generally more deep-seated, and quickly develop a connection with the phloem tissue, usually through a much enlarged ray. As the sphaeroblast increases its size by the development of an apical meristem and by fusion with new meristematic centres near its circumference, considerable stresses are set up which apparently cause the bark to lift away from the central woody cylinder just below the sphaeroblast. This leads to the formation of a pad of callus-like tissue with radially elongated elements between the base of the sphaeroblast and the xylem. In Fig. 2 (iib and iii) this callus-like

tissue, in which a continuation of the parenchymatous rays can just be distinguished, is marked with a C. In this region a new vascular cambium, represented in Fig. 2 (iv) by the broken thick black line, is differentiated. In Fig. 3d the apical meristem of the pro-sphaeroblast and its basal pad of callus-like tissue can be clearly seen. The crescent-shaped breaks which occurred in the callus pad during sectioning mark the position of the newly differentiating vascular cambium.

The final stages in the formation of a sphaeroblast shoot are shown in Fig. 5. The central woody cylinder laid down before disbudding and the adjoining triangular area of new tissue formed from the base of the sphaeroblast can be seen clearly. At first this newly-formed connecting tissue, being made of unlignified callus-like cells, is very soft and at this stage sphaeroblast shoots easily break out. Later the cambium differentiated in the connecting tissue joins up with that of the mother stem, and gradually normal growth is resumed all over the disbudded stem, the connection of which with the sphaeroblast shoot is strengthened by new tissue laid down normally from the connecting cambium.

A proportion of sphaeroblasts (60 per cent. according to Wellensiek, and approximately 40 per cent. according to Hatcher and Garner) do not develop shoots. Such sphaeroblasts develop to form the typical woody bark inclusions so extensively described in the older literature. Isolated vascular elements differentiate within the pro-sphaeroblast (Fig. 2 (iia)) and a vascular cambium develops around it, giving rise to normal xylem and phloem tissues with annual rings. The sphaeroblast shown in Fig. 4, which occurred naturally in a pear stem, shows four annual rings. The several growth centres from which it was initiated can be clearly seen in the middle. According to Krick (1891), wood elements in the centre and elements of the oldest phloem on the outside of the sphaeroblast exhibit the characters of primary xylem and phloem, and this is of interest in view of the adventitious origin of the sphaeroblasts. Naturally occurring sphaeroblasts in apple, pear, holly, fig and laburnum showed a similar construction. In laburnum a fully lignified sphaeroblast with an adjoining new meristematic centre was found, and detached sphaeroblasts of this species were able to produce shoots when placed in peat in a greenhouse. Fully lignified sphaeroblasts of holly can likewise produce very small shoots under natural conditions. In many instances such shoots actually arise from new meristematic centres which, in some species, form freely close to existing sphaeroblasts and rapidly make contact with them. In one two-year-old laburnum sphaeroblast a shoot originated from a latent bud in the centre of the sphaeroblast. Latent buds, according to MacDaniels (1953), are laid down by the primary meristem and, during their dormant state in the mother stem, they do not increase their mass, but retain their position in the bark by annual cambial growth of the bud trace.

DISCUSSION.

Three points of interest arise from these investigations :—(1) the exact location of the sphaeroblast primordia, (2) the possible correlation between rooting capacity and the ontogeny of organs, and (3) the effect of disbudding on the anatomy and growth processes of apple stems.

The early literature suggests that sphaeroblast-like structures occurring naturally in different woody species originate, (a) from latent buds developed from the pith but secondarily abscissed from the xylem of the mother stem, (b) from " bud

embryos " or adventitious buds inhibited in their development, or (c) from independent adventitious formations. In disbudded Crab C stems, sphaeroblasts arose in the last mentioned way, by the adventitious formation of meristematic centres in the cortex. Both Stoutemyer (1937) and Dermen (1948) agreed that the origin was adventitious, but they believed the meristematic initials to be more deep-seated than the cortex. Dermen thought that sphaeroblasts were initiated in the outer phloem, and Stoutemyer that they were formed from a cambium surrounding a group of phloem fibres. While such groups were occasionally found as inclusions in developing sphaeroblasts, there was no evidence that they acted as the centres of initiation. Shoots arose from sphaeroblasts either immediately by transformation of one of the meristematic centres into an apical meristem, or after lignification, usually from a new meristematic centre near the outer edge of the sphaeroblast, or more rarely from a latent bud laid down in its centre. In every case sphaeroblast shoots arose from a " massed " meristem originating adventitiously in a primary tissue and unconnected with the vascular cambium or with tissues derived from it.

The origin of sphaeroblast shoots is of more than academic interest in connection with Stoutemyer's views on juvenility, rooting capacity and the origin of organs. Stoutemyer (1937) has shown, and Garner and Hatcher (1948) have confirmed, that cuttings taken from shoots which arose directly on roots have an increased rooting capacity. Although he was aware that the etiolated base of such shoots would in itself improve the rooting, Stoutemyer believed that the greatly increased rooting ability of such shoots was due to their adventitious origin from newly formed bud initials in the parenchymatous cells of the cortex. The present investigation shows that sphaeroblast shoots arise from stems in an ontogenetically similar manner. That the histological origin, rather than the nutritional status, of shoots is of primary importance is further demonstrated by Stoutemyer's observation that watersprouts, formed from latent buds in severely pruned or decapitated trees, did not show an increased rooting capacity, although in many respects their nutritional status, resulting from the severe disturbance of the root/shoot ratio after decapitation, must be very similar to that of sphaeroblast shoots on disbudded stems. Only when the watersprouts grew from latent buds at the base of a seedling tree, i.e. had arisen from tissue formed by a *newly originated* meristem shortly after its initiation, did they show an increased capacity to form roots.

It appears that two ontogenetic features may be of importance with regard to rooting potential. One is the *de novo* origin of the meristem, and the other is its shape. Shoots from roots, latent buds and sphaeroblasts arise from an apical or " massed " meristem, as opposed to a cambial or 2-dimensional one. The work described by Loomis (1937) indicates that growth in these two types of meristem is to some extent based on different processes and is in some measure antagonistic, the one to the other. This appears to be true also for the apple. In Crab C stems normal cambial growth depended on the presence of buds and did not occur in disbudded trees. This confirms the observation of Wareing (1950) that in diffuse porous trees, of which the apple is an example, no cambial growth occurred following decapitation or disbudding. Removal of buds does not, however, suppress all forms of growth but results, on the contrary, in increased activity of other meristematic centres. In the apple the first response to decapitation, or indeed to severe pruning, is the transference of growth from the cambium to the preformed apical meristems of latent buds, which grow out, as occurred in the experiments of MacDaniels (1953).

If the latent buds are allowed to grow, normal cambial activity is resumed and no sphaeroblasts are formed. If, however, the latent buds also are removed as they appear, as was done by Hatcher and Garner (1955), then the growth processes are transferred to yet another tissue, the cortex, with inception of meristematic centres and sphaeroblast formation. As soon as sphaeroblast shoots develop, normal cambial activity is resumed and no further sphaeroblasts emerge, suggesting that cortical activity has also stopped. This finding suggests a way in which the production of new meristematic centres may be encouraged and, if Stoutemyer's hypothesis is correct, one in which plants may be brought into a state of increased rooting potential.

SUMMARY.

The origin and development of sphaeroblasts and of sphaeroblast shoots arising on disbudded stems of Crab C apple are described. Some measurements were made of anatomical changes induced by the disbudding treatment and the bearing of these observations on the growth processes of the apple, and their possible significance in relation to the improved rooting of stems, are discussed.

ACKNOWLEDGMENTS.

The authors acknowledge gratefully the help of Mr. R. J. Garner and Dr. E. S. J. Hatcher who suggested this investigation and provided the material for it, and without whose enthusiastic support it would not have taken place. We also thank Miss J. Hay who took the photographs.

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THE EFFECTS OF MOLYBDENUM DEFICIENCY AND MINERAL TOXICITIES ON CROPS IN ACID SOILS

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THE nature and interaction of the nutritional factors operating in acid soils have been studied by Hewitt (5) and the symptoms developed by plants grown in sand culture have been described by Hewitt and Jones (3). In field experiments conducted in Devon and Somerset on four soils with pH ranging from 4·4 to 5·1, Plant (8) was able to determine the factors deleterious to oats, barley, potatoes, kale and sugar beet; these included deficiencies of magnesium and molybdenum and excesses of manganese and aluminium.

The present paper records the occurrence of molybdenum deficiency and mineral toxicities in cauliflower and lettuce grown in rotation from 1950 to 1953 at Bromham, Wiltshire, and in a selection of brassicas grown between 1949 and 1953 at sites in Wiltshire, Somerset and Glamorgan.

I. CAULIFLOWER.

A number of experiments in sand culture and in the field have shown cauliflower to be highly susceptible to both molybdenum deficiency and manganese toxicity. Molybdenum deficiency can induce the condition known as " whiptail ", which may, however, occur under various environmental conditions not necessarily involving a shortage of molybdenum (9).

EXPERIMENTAL.

An experimental area of 36 × 24 sq. yd. was obtained by ploughing in 1950 a permanent pasture which formed part of a market garden at Bromham, Wiltshire, and in which severe incidence of whiptail in cauliflower had previously been observed. The soil, derived from the Lower Greensand, had a pH of 5·2.

The area was divided into 24 plots to give 3 randomized blocks of 8 treatments (Table I) designed to correct molybdenum or calcium deficiencies or to raise the soil pH.

Applications of gypsum, ground limestone and sodium molybdate were made in 1950 ; the dressings of limestone and sodium molybdate were repeated in 1953 when it had become apparent from pH measurements (Table I) and the incidence of whiptail (Table II) that the effects of the first applications had been transient. It will be seen from Table I that the pH of the unlimed plots fell by about 0·5 during the course of the experiment.

Seedling treatment with sodium molybdate (Treatment 8) was made annually in a bed adjacent to the plots when seedlings were 4 weeks old ; seed for the remaining treatments was sown in one of the control plots in order to obtain seedlings deficient in molybdenum. The variety Majestic was used throughout. A basal dressing of an NPK* mixture (7:7:7) was given annually to all plots at the rate of 8 cwt. per acre.

* N, P_2O_5 and K_2O.

Determinations of water-soluble molybdenum showed the contents of the gypsum and limestone to be 0·006 and 0·018 p.p.m. respectively. The amounts applied per acre in the gypsum and the two limestone treatments were therefore only 0·018, 0·054 and 0·108 gm. respectively, quantities too small to correct molybdenum deficiency in the crop.

TABLE I.

Treatments and soil pH ; Bromham, 1950–53.

Treatment	Rate per acre	Date applied	pH 28.10.50	pH 5.3.53	Applied 5.3.53	pH 1.9.53
1. Control	—	—	5·2	4·6	—	4·5
2. Gypsum	3 tons	20.2.50	4·9	4·5	—	4·5
3. Gypsum Sodium molybdate ..	3 tons 2 lb.	20.2.50 27.2.50	5·0	4·6	— 2 lb.	4·6
4. Ground oolitic limestone ..	3 tons	20.2.50	6·3	5·6	3 tons	7·0
5. Ground oolitic limestone ..	6 tons	27.2.50	6·4	6·1	6 tons	7·1
6. Sodium molybdate ..	2 lb.	27.2.50	5·4	5·0	2 lb.	5·0
7. Sodium molybdate ..	4 lb.	27.2.50	5·2	4·9	4 lb.	4·8
8. Sodium molybdate ..	1 oz. per 10 sq. yd.	Annually 1–15 May	5·5	4·9	—	4·8

Observations of leaf and root symptoms were made throughout the season ; the cauliflower heads were graded in September and October (Table II) and determinations of molybdenum, manganese, nitrate and ascorbic acid were made on leaf samples collected twice in each season.

RESULTS.

Visual symptoms.

Hewitt (3) has described the syndrome of molybdenum deficiency in cauliflower growing in sand culture. In the field the syndrome shows wide variation. The general form of whiptail induced by molybdenum deficiency has been described by Plant (6), but symptoms in the early stages of growth had not then been observed ; in the present experiment they were identical with those described by Hewitt.

The first symptoms were noticed in the seedling cauliflowers one month after sowing ; they consisted of chlorosis, cupping and elongation of the leaves (Fig. 1). The seedlings occasionally showed some recovery before being planted out but the symptoms recurred in the gypsum and control treatments. The younger chlorotic leaves had either shrivelled and fallen, or turned green, by the end of August, whereas the new leaves developed the straplike and malformed petioles characteristic of whiptail. At harvest the whiptail plants exhibited a wide range of symptoms including blind or partially blind growing-points (Fig. 2).

No manganese toxicity effects were apparent in 1950–52 except for occasional necrotic spots on the leaves in the early stages of growth ; these symptoms did not

persist. In 1953, however, symptoms of the toxicity, characterized by marginal incurling, leaf malformation and necrotic spotting, appeared in the seedlings and transplants in all treatments not receiving lime, and persisted throughout the season ; many plants in the control and gypsum treatments had died by the end of June. The toxicity was shown very markedly by plants receiving molybdenum (Fig. 3).

Yields.

At harvest the cauliflower heads were graded and a count of whiptail plants was made ; this classification had to be modified in 1953 because of the incidence of manganese toxicity and a reduction in the number of marketable heads. Table II gives the percentages of the different market grades obtained in each of the four years.

It will be seen that from 1950 to 1952 there was a progressive decline in the number of marketable heads and an increase in the incidence of whiptail. Poor yields were obtained throughout from the control plots and from the gypsum treatment which increased the occurrence of whiptail. Fairly consistent yields were given by the molybdate treatments until 1953, when marketable heads were obtained only from the limed plots and from plants receiving molybdate in the seed bed.

Leaf analyses.

Determinations of molybdenum, manganese, nitrate and ascorbic acid were made twice in each season on leaves of intermediate age taken midway between the outside and centre of the plant ; the petioles were removed and only the laminae used for analysis. The values are given in Table III.

Molybdenum. It will be seen that in 1950 the level of molybdenum was low (0·09 p.p.m.) in leaves from the control plots and slightly lower in those from the gypsum plots, where the incidence of whiptail was higher ; significantly higher levels were present in leaves from the lime and soil molybdate treatments (3–7 of Table III).

During the following three years there was a gradual decline in the levels in samples from all plots ; the increases due to molybdate had lost their significance by the end of 1951 and the effect of lime was barely significant by the beginning of 1952. Reapplication of molybdate in 1953 had no effect on the levels, but significant increases due to re-liming were apparent in samples taken in September of that year.

In view of the higher yields given by seedlings treated with molybdate in the seed-bed (Table II), it is of interest that at no time was the molybdenum level in the leaf samples greater than in those from the untreated controls. There had been a concentration of 40 p.p.m. molybdenum in the leaves of these seedlings at the time of transplanting.

Manganese. The levels of manganese found in the 1951 samples were generally low and the effect of liming was not marked. In 1953 there were high levels in all treatments not receiving lime ; the highest value (2,375 p.p.m.) was found in plants from the gypsum treatment.

Nitrate. Nitrate was determined in fresh material in 1950 and in the dried bulk samples in 1951 and 1952. The values were positively correlated with the severity of whiptail. Significantly higher levels were obtained throughout from plots receiving gypsum (Treatment 2) and the reduction due to the addition of molybdate (Treatment 3) was well-marked. Significantly high values were also obtained in leaves taken from the control plots from 1951 onwards.

TABLE II.

Percentage of marketable and whiptail cauliflowers at harvest; Bromham, 1950–53.

Treatment	1950					1951					1952					1953			
	G*	L	M	U	W	G	L	M	U	W	G	L	M	U	W	L & M	U	Mn excess	W
1. Control	5	30	35	6	24	0	17	13	36	34	0	9	15	16	60	0	12	88	0
2. Gypsum	1	10	10	0	79	0	23	15	22	40	0	7	9	24	60	0	10	90	0
3. Gypsum + molybdate ..	8	28	41	19	4	0	42	25	27	6	0	18	30	21	31	0	13	87	0
4. Limestone, 3 tons	7	28	35	30	0	0	32	23	28	17	0	18	31	18	33	58	32	0	10
5. Limestone, 6 tons	5	28	40	27	0	0	33	25	38	4	0	16	39	20	25	55	37	0	8
6. Molybdate, 2 lb.	4	26	47	23	0	0	37	24	34	5	0	20	27	25	28	0	7	93	0
7. Molybdate, 4 lb.	0	32	45	23	0	0	36	31	29	4	0	18	18	25	39	0	5	95	0
8. Molybdate to seed bed ..	5	30	39	26	0	0	35	25	34	6	0	36	39	17	8	53	38	9	0

* G, giant (4 heads per bushel box); L, large (6 heads per bushel box); M, medium (12 heads per bushel box); U, ungraded (small heads); W, whiptail.

TABLE III.

Leaf analyses ; cauliflower, Bromham.

Treatment	Sampled 16.8.50				Sampled 3.8.51					Sampled 13.8.52				Sampled 8.8.53		
	Symptoms*	Dry wt.	Fresh wt.		Symptoms*	Dry wt.			Fresh wt.	Symptoms*	Dry wt.		Fresh wt.	Symptoms*	Dry wt.	
		Mo p.p.m.	NO_3 p.p.m.	Ascorbic acid mg./100 gm.		Mo p.p.m.	Mn p.p.m.	NO_3 per cent.	Ascorbic acid mg./100 gm.		Mo p.p.m.	NO_3 per cent.	Ascorbic acid mg./100 gm.		Mo p.p.m.	Mn p.p.m.
1. Control	m	0·10	1,920	173	s	0·06	121	2·03	188	s	0·05	2·93	152	Mn+	*O*	850
2. Gypsum	s	0·06	4,150	130	s	0·05	236	3·33	164	s	0·08	3·81	135	Mn+	*O*	2,260
3. Gypsum+molybdate	t	0·09	1,420	177	n	0·17	159	1·44	188	m	0·11	1·52	176	Mn+	0·07	990
4. Limestone, 3 tons	n	0·22	1,390	190	n	0·14	64	1·39	181	m	0·09	1·94	179	t	0·07	140
5. Limestone, 6 tons	n	0·39	1,310	191	n	0·24	119	1·69	183	m	0·07	1·94	180	t	0·08	125
6. Molybdate, 2 lb.	n	0·37	1,310	208	n	0·19	101	1·42	188	m	0·11	1·70	191	Mn+	0·04	1,680
7. Molybdate, 4 lb.	n	0·86	1,570	194	n	0·22	176	1·49	182	m	0·09	2·39	150	Mn+	0·06	1,640
8. Molybdate to seed bed	n	0·09	1,510	176	n	0·11	116	1·39	193	m	0·08	1·97	171	Mn+	0·04	630
S.E.		±0·09	±376	±5·4		±0·025	±36	±0·24	±8·2		±0·017	±0·35	±8·8			
	Sampled 29.9.50				Sampled 25.9.51					Sampled 22.9.52				Sampled 21.9.53		
1. Control	m	0·08	1,880	129	s	0·05	176	2·06	154	s	0·03	0·98	177	Mn+	*O*	*O*
2. Gypsum	s	0·06	2,990	106	s	0·03	151	1·91	156	s	0·04	1·28	175	Mn+	0·03	2,375
3. Gypsum+molybdate	t	0·29	2,250	147	t	0·07	76	0·70	191	m	0·06	0·39	201	Mn+	0·05	1,090
4. Limestone, 3 tons	n	0·53	1,670	127	t	0·12	55	0·99	206	m	0·06	0·32	184	t	0·13	210
5. Limestone, 6 tons	n	0·81	1,580	131	t	0·22	95	0·82	203	m	0·08	0·40	200	t	0·20	260
6. Molybdate, 2 lb.	n	0·44	1,170	145	t	0·07	116	0·75	196	m	0·04	0·40	194	Mn+	0·03	1,240
7. Molybdate, 4 lb.	n	0·91	1,390	145	t	0·06	103	0·66	192	m	0·04	0·63	194	Mn+	0·07	1,030
8. Molybdate to seed bed	n	0·08	1,500	138	t	0·06	123	0·67	187	t	0·03	0·55	182	Mn+	0·03	1,510
S.E.		±0·07	±400	±7·0		±0·019	±27	±0·28	±6·79		±0·011	±0·16	±7·2			

* Incidence of whiptail : s, severe ; m, moderate ; t, trace ; n, none. Mn+, Mn toxicity. *O*, insufficient material.

Ascorbic acid. Determinations of ascorbic acid were made in fresh material taken from the bulked samples used for the estimation of molybdenum and manganese. The levels were consistently lowest in plants from the gypsum treatment, the difference being highly significant in the first year of the experiment and becoming less noticeable as whiptail began to appear in other treatments.

II. VARIOUS BRASSICAS.

Cabbage, brussels sprouts and savoy were included with cauliflower in the experiments made at Bromham in 1953 (Part I). Marrowstem kale (*Brassica oleracea acephala*), which had proved a sensitive indicator of mineral deficiencies and excesses in trials carried out by the writer (8) in 1948–51 on four acid soils, including one derived from Old Red Sandstone at Portbury, Somerset, was included with cabbage, cauliflower, brussels sprouts and rape in comparative trials made in 1951 and 1952 on the same site at Portbury. Cabbage, cauliflower, brussels sprouts and savoy were also grown together in 1949 on an acid soil derived from Old Red Sandstone drift in the Gower Peninsula, Glamorgan.

Experimental.

Soil pH at the three sites was :

1. The Gower 1949 ; continuous cropping since 1946 ; pH 4·8.
2. Portbury 1951 ; „ „ „ 1941 ; pH 4·0.
3. Bromham 1953 ; „ „ „ 1950 ; pH 4·6.

At the Gower and Bromham 3 blocks of the following 4 treatments, arranged at random in parallel strips of 6×4 sq. yd., were used. The brassicas were sown in rows at right angles to the length of the plot, their order being randomized afresh for each block.

1. Control. No treatment.
2. Gypsum. 3 tons per acre.
3. Ground limestone. 3 tons per acre.
4. Sodium molybdate. 4 lb. per acre.

At Portbury an application of 3 tons ground dolomite per acre replaced the molybdate treatment.

All plots received an annual application of 8 cwt. mixed NPK fertilizer* (7:7:7) per acre.

The following varieties of brassica were used :—cabbage, Rapidity ; cauliflower, Majestic ; brussels sprouts, Cambridge No. 1 ; kale, Marrowstem ; rape, English Broad Leaved ; savoy, Best of All.

Observations of habit of growth and vigour and of the symptoms appearing on leaves and roots were made throughout the season. Determinations of molybdenum and manganese were made in leaf samples taken once in each season ; measurements of nitrate and ascorbic acid were also made in the Portbury samples.

Results.

Symptoms and analytical data.

The symptoms developed by the crops which were grown at all three sites are listed in Table IV, together with analytical data for the leaf samples. More detailed

* See footnote p. 163.

TABLE IV.

Soil pH, symptoms and leaf analyses ; brassicas.

Treatment	Soil pH	Cabbage			Cauliflower			Brussels sprouts			Savoy		
		Symptoms*	Dry wt. Mo p.p.m.	Dry wt. Mn p.p.m.	Symptoms	Dry wt. Mo p.p.m.	Dry wt. Mn p.p.m.	Symptoms	Dry wt. Mo p.p.m.	Dry wt. Mn p.p.m.	Symptoms	Dry wt. Mo p.p.m.	Dry wt. Mn p.p.m.
1. *The Gower, 3.8.49*													
Control ..	4·8	Mo—	0·08	815	Mo—	0·06	660	Mo—	0·10	486	Mo—	0·13	725
Gypsum ..	5·2		0·11	387		0·10	253		0·16	320		0·19	263
Limestone ..	7·1		0·16	272		0·12	172		0·13	241		0·19	148
Molybdate ..	5·1		0·28	703		0·22	298		0·20	405		0·36	406
2. *Portbury, 30.7.52*													
Control ..	4·0	Mn+, Al+	0·13	906	Mo—, Mn+, Al+	0·11	743	Mo—, Mn+, Al+	0·16	1,037	not grown		
Gypsum ..	4·0	Mn+, Al+	0·13	724	Mo—, Mg—, Mn+, Al+	0·10	456	Mo—, Mg—, Mn+, Al+	0·10	818			
Limestone ..	6·5		0·40	69		0·26	80		0·37	93			
3. *Bromham, 8.8.53*													
Control ..	4·6	Mo—, Mn+	0·02	1,110	Mo—, Mn+	0·03	850	Mo—, Mn+	0·02	1,110	Mo—, Mn+	0·05	980
Gypsum ..	4·5	Mo—, Mn+	0·03	1,540	Mo—, Mn+	0·03	2,260	Mo—, Mn+	0·05	1,700	Mo—, Mn+	0·03	2,970
Limestone ..	7·1		0·11	235		0·08	125		0·04	180		0·13	610
Molybdate ..	4·6	Mn+	0·13	1,070	Mn+	0·06	1,640	Mn+	0·08	850	Mn+	0·16	680

*Mo—, molybdenum deficiency ; Mn+, manganese toxicity ; and so on.

data, including crop yields and nitrate and ascorbic acid levels in the leaf samples, were obtained in the 1951 experiment at Portbury and are set out in Table V.

TABLE V.

Yields and leaf analyses ; brassicas, Portbury, 1951.

Treatment	Symptoms*	Yield tons/ acre	Dry wt.			Fresh wt.
			Mo p.p.m.	Mn p.p.m.	NO_3 per cent.	Asc. acid mg./100 gm.
			26.7.51	26.7.51	26.7.51	26.7.51
	Cabbage					
Control	Mn+, Al+	7·5	0·10	1,398	1·12	126
Gypsum	Mn+, Al+	15·2	0·13	663	0·73	117
Limestone		32·5	0·37	89	0·56	118
Dolomite		34·0	0·44	53	0·64	135
	S.E.	±1·98	±0·049	±103	±0·13	±6·3
	Cauliflower					
Control	Mo−, Mn+, Al+ ..	5·5	0·12	2,121	0·76	185
Gypsum	Mo−,Mg−,Mn+,Al+	4·7	0·09	884	0·86	188
Limestone		14·7	0·30	99	0·66	208
Dolomite		15·0	0·32	92	0·56	214
	S.E.	±1·04	±0·030	±194	±0·07	±10·1
	Brussels sprouts					
Control	Mo−, Mn+, Al+ ..	7·5	0·16	1,137	1·11	153
Gypsum	Mo−,Mg−,Mn+,Al+	12·7	0·14	855	1·32	154
Limestone		21·5	0·60	86	1·19	183
Dolomite		19·7	0·64	91	1·21	191
	S.E.	±1·88	±0·04	±119	±0·10	±11·1
	Kale					
Control	Mn+, Al+	11·5	0·18	1,522	1·25	158
Gypsum	Mg−, Mn+, Al+ ..	13·5	0·26	666	0·98	155
Limestone		28·2	1·01	80	0·90	194
Dolomite		27·7	0·90	92	0·87	186
	S.E.	±1·50	±0·086	±139	±0·11	±14·8
	Rape					
Control	Al+	7·2	0·28	745	1·04	138
Gypsum	Mg−, Al+	9·2	0·03	480	0·88	146
Limestone		12·5	1·01	96	0·76	141
Dolomite		13·0	0·94	86	0·83	137
	S.E.	±0·88	±0·024	±43	±0·07	±4·0

* As in Table IV.

The Gower. It will be seen that molybdenum deficiency appeared in all crops grown on the control plots (Fig. 4), but in no others. Unlike its effect with cauliflower at Bromham, where it reduced soil pH and intensified the incidence of whiptail (Tables I and II), gypsum raised the pH and produced normal plants containing an increased concentration of molybdenum and a reduced level of manganese. Lime had a similar though more marked effect on both molybdenum and manganese levels, while the molybdate treatment gave normal plants containing a relatively high level of molybdenum.

Bromham. At Bromham normal plants were produced only by the lime treatment. In both the control and gypsum plots, a mixed syndrome of molybdenum

deficiency and manganese toxicity appeared while manganese toxicity occurred alone on the plots treated with sodium molybdate. It will be seen that gypsum had no significant effect on the concentrations of molybdenum in the plants but caused a marked increase in the manganese levels, particularly in cauliflower and savoy, where the concentrations were almost three times as high as those in the control plants. The reduction in manganese uptake caused by lime was very marked, but the increases in molybdate level due to the lime and molybdate treatments were not as pronounced as at the Gower.

Portbury. In both 1951 and 1952, symptoms of severe manganese and aluminium toxicities were evident on plants on the control and gypsum plots. Symptoms of molybdenum deficiency were easily seen in cauliflower (whiptail) and in brussels sprouts (Fig. 5), but in the other brassicas any symptoms of molybdenum deficiency were effectively masked by the toxicity effects.

Manganese toxicity, characterized by incurling of the laminae and chlorosis of the leaf margins followed by necrotic spotting, appeared on cabbage, cauliflower, brussels sprouts (Fig. 5) and kale, but not on rape, which Hewitt (2) has shown to be tolerant of manganese excess. Rape showed very clearly the effects of aluminium toxicity, consisting of a purpling of petioles and leaves suggestive of phosphate deficiency. Similar symptoms occurred, together with symptoms of manganese toxicity, in the other brassicas. The difficulty of diagnosing the dominant deficiency or excess when mixed symptoms are present is illustrated in Fig. 5 which shows leaves of brussels sprouts chosen for their specific symptoms. Marbling, indicative of magnesium deficiency, appeared in the older leaves of rape, kale, brussels sprouts and cauliflower, but only on plots receiving gypsum.

Yields and leaf analyses.

As has already been stated, the only available data under this heading are those obtained in the Portbury experiment of 1951 and summarized in Table V. Regarding yields, it will be seen that dolomite and limestone were equally effective in correcting molybdenum deficiency and manganese and aluminium excesses and in producing normal yields. Except for cauliflower, yields from the gypsum plots were higher than from the controls, the increases for cabbage and brussels sprouts being significant.

Dolomite and limestone showed effects of the same order in greatly increasing the molybdenum status of leaves and in decreasing their content of manganese. Though it had no consistent effect on molybdenum levels, gypsum significantly depressed the levels of manganese in all types of brassica. The relatively low levels of manganese in rape leaves will be noticed, as also the high levels of molybdenum present in the leaves of both rape and kale.

Significantly high levels of nitrate were present in the leaves from the control plots of cabbage, kale and rape and from the gypsum plots of cauliflower. Significant differences in ascorbic acid content were obtained only with cauliflower and brussels sprouts, where the highest levels were given by the lime and dolomite treatments.

III. LETTUCE.

The susceptibility of lettuce to molybdenum deficiency in water cultures was shown by Brenchley and Warington (1). Its cultivation is usually carried out on highly fertile soils, but Wilson (10) observed a chlorosis in lettuce growing on an acid

Australian soil of pH 5·2 and found that this could be corrected by spraying the leaves with solutions of sodium molybdate. Field trials made at Bromham between 1951 and 1953 have shown some striking responses to applications of either sodium molybdate or lime (Plant, 7).

Experimental.

Lettuce was grown during three successive years on the plots used for the cauliflower trials already described (Part I), omitting the seed bed treatment (Treatment 8).

The 1952 crop was discarded because of an attack of greenfly and infection with *Botrytis cinerea*. The variety Borough Wonder was sown in 1951 (1.3.51) under Dutch lights adjacent to the experimental plot ; planting out commenced 29.3.51 and plants were cut for market between 20.6.51 and the end of the month. In 1953 the variety Improved Trocadero was sown (22.5.53) in drills on the experimental plots and the seedlings were subsequently thinned. The hearted lettuces on the limed plots were cut during the latter half of July.

Observations of leaf symptoms and habit of growth were made throughout the growing season ; leaf samples for the determination of molybdenum, manganese and ascorbic acid were taken a few weeks prior to harvesting ; yields of hearted lettuces were recorded.

Results.

Symptoms.

In 1951 and 1952 severe symptoms of molybdenum deficiency occurred in the control and gypsum treatments. The deficiency was not apparent until early May, when the growth rate began to increase ; by the end of May a large proportion of the affected plants had died.

In the early stages deficient plants were chlorotic and stunted ; the laminae failed to develop and the leaves remained ovate rather than round as in a healthy plant. Chlorosis of the leaf was followed by necrosis of the margin ; the leaf was of papery texture and almost translucent, while the necrotic areas were light brown (Fig. 6). Where the deficiency was severe the plants failed to heart and eventually shrivelled and died.

Severe molybdenum deficiency also appeared in the control and gypsum treatments in 1953, when there were also slight symptoms in plots receiving molybdenum but none in those treated with limestone (Table VI). The symptoms were, however, complicated by the effect of manganese toxicity in all the unlimed plots. This toxicity was characterized by a golden yellow margin which extended round all leaves but was most noticeable on the lowest and largest ones (Fig. 7). Where the plant had sufficient molybdenum to maintain a leaf with a green centre, the marginal effect was most striking ; when, however, the plant suffered both from molybdenum deficiency and from manganese excess, the leaves were chlorotic with distinct yellow margins.

Yields.

It will be seen that in 1951 the yields from treatments receiving either lime or sodium molybdate were about double those of the control and gypsum treatments. The lowest yield of 24 cwt. per acre, obtained on the gypsum plots, was nearly

PLATE I

Fig. 1.

Molybdenum deficiency in 12-weeks-old cauliflower, showing interveinal chlorosis and the beginning of leaf malformation. Bromham, 1950.

Fig. 2.

Molybdenum deficiency in mature cauliflower, showing whiptail effects and atrophy of growing point. Bromham, 1950.

Fig. 3.

Severe manganese toxicity in mature cauliflower, showing necrotic spotting of incurled leaf margins. This plant received molybdenum in the seed bed. Bromham, 1953.

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FIG. 4.

Molybdenum deficiency in 12-weeks-old brassicas. The Gower, 1949.

1. Cabbage ; lack of hearting.
2. Savoy ; lack of hearting.
3. Brussels sprouts ; reduction and cupping of laminae.
4. Cauliflower ; malformation of laminae (whiptail).

FIG. 5.

Leaves of brussels sprouts. Portbury, 1951.

1. Syndrome of molybdenum deficiency predominant.
2. Syndrome of manganese toxicity predominant : note necrotic spotting of incurled margins.

1 2

FIG. 6.

Molybdenum deficiency in lettuce. Bromham, 1951.

1. Molybdenum deficient lettuce, showing translucent ovate leaves with necrotic margins and failure to heart.
2. Normal lettuce from treatment with 4 lb. sodium molybdate per acre.

FIG. 7.

Manganese toxicity in lettuce, showing marginal chlorosis of the older leaves. Bromham, 1953.

TABLE VI.

Yields and leaf analyses ; lettuce, 1951 and 1953.

Treatment	Soil pH	Symptoms*	Yield† cwt./acre	Dry wt.		Fresh wt.
				Mo p.p.m.	Mn p.p.m.	ascorbic acid mg./100 g.
1951						
1. Control	5·2	Mo— —	32	0·06	—	50
2. Gypsum	4·9	Mo— —	24	0·06	—	51
3. Gypsum+molybdate	5·0		62	0·10	—	57
4. Limestone, 3 tons ..	6·3		59	0·09	—	65
5. Limestone, 6 tons ..	6·4		56	0·14	—	60
6. Molybdate, 2 lb. ..	5·4		45	0·08	—	63
7. Molybdate, 4 lb. ..	5·2		64	0·08	—	55
L.S.D. at 5% level ..				0·07		17
1953						
1. Control	4·5	Mo— —, Mn++	Failure‡	0·07	3,330	29
2. Gypsum	4·5	Mo— —, Mn++	,,	0·06	4,060	29
3. Gypsum+molybdate	4·6	Mo—, Mn++	,,	0·04	3,640	56
4. Limestone, 3 tons ..	7·0		50	0·04	370	35
5. Limestone, 6 tons ..	7·1		60	0·04	310	31
6. Molybdate, 2 lb. ..	5·0	Mo—, Mn++	Failure	0·04	4,220	45
7. Molybdate, 4 lb. ..	4·8	Mo—, Mn++	,,	0·06	3,270	63
L.S.D. at 5% level ..						42

* Mo—, slight molybdenum deficiency ; Mo— —, severe molybdenum deficiency ; Mn++, severe manganese toxicity.

† Means for 3 plots of each treatment.

‡ None of the crop marketable.

trebled by the addition of 4 lb. molybdate per acre. In 1953 a marketable crop was obtained only on the limed plots, where the yield was of the same order as that obtained in 1951 ; all other treatments gave plants that were too small to market.

Leaf analyses.

Leaf samples were collected on 6.6.51 and 7.7.53. Determinations of ascorbic acid were made on fresh material taken from bulked samples in which molybdenum and manganese were later determined. The values are given in Table VI.

Molybdenum. It will be seen that, although in 1951 the lowest molybdenum levels (0·06 p.p.m.) were found in the leaves from the two treatments (control and gypsum) in which molybdenum deficiency appeared, the differences between treatments were small. Only in leaves from the higher lime treatment was the level significantly greater than in the control. In 1953 all levels were low, the concentration (0·04 p.p.m.) in the healthy green plants produced on the limed plots being no higher than in the stunted plants from the remaining treatments.

Manganese. Manganese was not determined in the 1951 samples. There was a high concentration (3–4,000 p.p.m.) in all the 1953 samples except in those from the two sets of limed plots.

Ascorbic acid. No significant differences due to treatment are apparent in the concentrations of ascorbic acid in the leaves. It will be noticed, however, that in 1953 the levels in the three molybdate treatments (Treatments 3, 6 and 7) were of the same order as those obtained in 1951, whereas there was a marked fall in the levels present in the leaves from other treatments, including lime.

DISCUSSION.

Cauliflower at Bromham.

Yields, symptoms and leaf analyses show very clearly the effect of the gradual fall in soil pH that occurred during the four years of experiment. In the first year whiptail was severe only where the pH had been depressed by gypsum ; there was then good response to soil treatment with molybdate, but the effect of this treatment declined in the two succeeding years, presumably because of the reduced availability of molybdenum at the lower pH.

By correcting the pH with lime it was possible throughout the first three years to counteract molybdenum deficiency more effectively than by the addition of molybdate to the soil. Only by addition of lime was a marketable crop obtained in the fourth year, when manganese toxicity led to failure in all other treatments except for those plants which had received molybdate in the seed bed.

The success of the seedling treatment is surprising in view of the marked symptoms of manganese toxicity and the low level (0·03 p.p.m.) of molybdenum in the leaves ; a high concentration of molybdenum in the young seedlings would appear to be a factor influencing the later development of the plants. It must be remembered that, although the concentration of molybdenum was no higher than in the untreated controls, the total content of molybdenum would be very much higher because of the greater size of the plants.

Except in the seedling treatment, low levels of molybdenum in the leaf are associated with the occurrence of whiptail which, as previously found by Hewitt *et al.* (4), is also characterized by an accumulation of nitrate and a low concentration of ascorbic acid in the leaf. It seems that these three criteria may serve to differentiate whiptail induced by molybdenum deficiency from pseudo-whiptail caused by the environmental checks described by Wiebosch *et al.* (9).

Various brassicas at three centres.

The results show first the effect of soil pH and cropping history on the symptoms developed at the three sites. On the soil of highest pH (the Gower) the brassicas showed only molybdenum deficiency, which could be corrected equally by sodium molybdate or limestone. On the more acid soil of Bromham there was a mixed syndrome of molybdenum deficiency and manganese toxicity, responding only to limestone. On the highly acid soil of Portbury the syndrome was further complicated by aluminium toxicity, also responding to treatment with limestone or dolomite.

The trials also show the effect of soil type. On both the soils derived from Old Red Sandstone (the Gower and Portbury), gypsum reduced the uptake of manganese and on one of them it corrected molybdenum deficiency and increased the concentration of molybdenum in the plants. On the soil derived from the Lower Greensand (Bromham), however, gypsum not only caused a marked increase in the levels of manganese in the leaves but also exaggerated the symptoms of molybdenum

deficiency in some of the brassicas. The variable behaviour of the different types of brassica is particularly well shown by the failure of rape to exhibit symptoms of manganese toxicity.

No regular correlation between molybdenum levels and the concentrations of nitrate and ascorbic acid in the leaves was found in the Portbury samples, possibly because of interference by manganese and aluminium toxicities.

Lettuce at Bromham.

This experiment, carried out on the same plots as were used for cauliflower at this centre, confirms the susceptibility of lettuce to molybdenum deficiency and shows that it is also sensitive to manganese excess. The effects of the gradual decline in pH of the unlimed plots were similar to those observed with cauliflower. In the first two years, when the symptoms were those of molybdenum deficiency, sodium molybdate was as effective as lime in countering the deficiency and in producing a normal crop. In the third year it only partially corrected the symptoms of molybdenum deficiency, and gave no marketable crop because of the parallel incidence of manganese toxicity. Only the lime treatments effectively prevented the latter.

Leaf analyses show very clearly the counter-effect of liming on manganese uptake ; they do not differentiate between the levels of molybdenum present in normal and deficient leaves nor do they suggest that lettuce is among the plants in which molybdenum deficiency is accompanied by a low level of ascorbic acid (4).

SUMMARY.

1. An account is given of field trials in which cauliflower, other brassicas and lettuce were grown for a varying number of years on three acid soils treated with limestone, dolomite, gypsum and sodium molybdate.

2. The symptoms developed by the test crops are described and depicted, and analytical data for leaf samples are given.

3. On the least acid soil (pH 4·8) only molybdenum deficiency was apparent ; on the soil of intermediate pH (4·6) a mixed syndrome of molybdenum deficiency and manganese toxicity appeared, whilst on the most acid soil (pH 4·0) the syndrome also included aluminium toxicity. When cauliflower and lettuce were grown on the same soil for four consecutive years, a gradual fall in soil pH led to a change from molybdenum deficiency to a syndrome of molybdenum deficiency and manganese toxicity.

4. Applications of lime and dolomite were equally effective in correcting both molybdenum deficiency and mineral toxicities ; they raised the molybdenum status of leaves and reduced their manganese content. While gypsum reduced the uptake of manganese from two soils derived from Old Red Sandstone and tended to alleviate molybdenum deficiency, it had the opposite effect on a soil derived from the Lower Greensand and gave plants containing up to 4,000 p.p.m. manganese.

5. Soil treatment with sodium molybdate was completely effective only on the least acid soil, but an unexpectedly high yield of cauliflowers showing symptoms of manganese toxicity was obtained on the soil of intermediate pH by spraying seedlings with molybdate in the seed bed.

6. Molybdenum deficiency was characterized by a high level of nitrate and a low level of ascorbic acid in the leaves of some brassicas but not of all.

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THE SPACING OF LETTUCE IN HEATED GLASSHOUSES

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THERE has been considerable controversy during the past few years regarding the spacing of lettuce in heated glasshouses. Some growers, particularly in Lancashire, have advanced the opinion that, although a wide spacing of about 9×9 square inches per plant will produce a large lettuce of good quality, nevertheless a close spacing of about 6×6 square inches per plant will produce a marketable though smaller lettuce, with resulting higher profit from the increased plant population.

This paper describes three experiments carried out over the period 1953–5 at the Luddington Experimental Horticulture Station, near Stratford-on-Avon. They were designed firstly to discover the effect of various spacings on the yield of marketable lettuce from a given area, and secondly to assess the corresponding relative profit.

Site.

The experiments were carried out in a 150 ft. by 30 ft. vinery-type glasshouse of traditional design, orientated north to south, which was heated by six runs of 4-inch diameter pipes fixed just above soil level. The heating was thermostatically controlled to maintain a minimum temperature of 45° F. in the glasshouse, the heat being conveyed by a low-pressure hot-water system. The lay-out of the experiments was such that each of the four blocks occupied one of the four beds in the house, this arrangement having been previously found to be the most efficient in eliminating environmental differences.

The soil had not long been used for glasshouse crops and winter lettuce had been grown in it only twice before 1953 ; it was in a high state of fertility. A soil analysis in February, 1955 gave the following figures :—pH, 7·58 (alkaline) ; pC, 3·33 (safe) ; available P_2O_5, 184 (high) ; available K_2O, 60 (high). The soil, belonging to the Pershore Series and consisting of a medium loam with coarse drift overlying Lias clay, had been improved by the addition of farmyard manure.

The variety of lettuce used was Cheshunt 5B and the seedlings were propagated from a late December sowing in seed-trays, being pricked out into small-size soil-blocks and planted out a month later. The crops matured approximately six weeks after planting. Soil preparation and manurial treatment were the same for all spacings.

1953 EXPERIMENT.

Four square spacings, 6×6, 7×7, 8×8 and 9×9 inches and four rectangular spacings, maintaining a constant inter-row distance of 9 inches but with distances in the row of 6, 7, 8 and 9 inches, were used. The plot size, including the guard

TABLE I.

Effect of spacing on grade and yield of lettuce ; 1953 crop.

Spacing (inches)	Number planted	Numbers				Weight (kg.)				Marketable weight (tons per acre)
		in grade*			Total	in grade			Total	
		24	30	36		24	30	36		
Square										
6×6	952	0	444	506	950	—	57·9	56·2	114·1	20·5
7×7	696	70	472	150	692	11·4	67·8	17·7	96·9	17·5
8×8	500	288	188	23	499	47·7	27·0	2·6	77·3	14·9
9×9	352	242	101	7	350	39·8	14·9	0·9	55·6	12·0
										†S.E., ±0·42
Rectangular										
9×6	544	158	347	38	543	30·1	45·7	4·6	80·4	16·9
9×7	464	226	228	8	462	38·4	34·3	1·0	73·7	15·6
9×8	400	263	130	6	399	46·3	19·1	0·7	66·1	14·1
9×9	352	263	83	6	352	47·0	12·5	0·8	60·3	13·0
										†S.E., ±0·44

* The grades 24, 30 and 36 refer to the number of lettuces packed in a standard crate.
† For differences of two means.

rows, was 90 square feet but the recorded area varied slightly with the spacings. The square and rectangular spacings were arranged in two separate 4×4 latin squares in the glasshouse, but comparison between treatments not in the same square was possible because the spacing 9×9 inches was common to both sets.

Results.

Table I shows the number of seedlings planted at each spacing, the number and weight, in grades, of lettuces cut at maturity and the total marketable weight converted to tons per acre *of growing area*. The evenness of the crop allowed a single harvest and all plants were cut on 1st April. Losses and wastage were both slight, 7 and 6 plants respectively out of a total of 4,260, and bore no obvious relation to spacing.

Each set of means shows a steady increase in marketable weight as the area per plant decreases and the closest spacing shows no sign of any falling off in this yield increase. From the graph of the yields in Fig. 1 it appears that the rectangular spacing has given a smaller increase in yield per unit decrease in area than the square spacing. A test of this has been made by fitting one straight line to all the rectangular set, and another to the three square spacings in the same range (i.e. excluding the 6×6) ; a difference in slope is obtained of $-0{\cdot}028 \pm 0{\cdot}020$ tons/acre per square inch, which is in the expected direction, though the difference is not significant ($P = 0{\cdot}05$) in this experiment.

From the market returns for this crop, averages (in shillings) per crate and per plant for the three grades 24, 30 and 36 were worked out, and from these averages and the data in Table I the estimated gross returns per acre for the various square spacings were evaluated. These are given in Table II.

Table II.

Market returns and estimated gross returns per acre ; 1953 crop.

Mean price (shillings)	Grade			Estimated gross returns per acre	
	24	30	36	Spacing	£
Per crate	15·2	16·6	15·5	6×6	4,240
				7×7	3,400
Per lettuce	0·63	0·55	0·43	8×8	2,900
				9×9	2,330

There is little difference in the average price per crate of the three grades. This has been found on other occasions, and since the weight of a crate of lettuce is roughly constant, it seems to be generally true that lettuce is in effect sold primarily by weight over a wide range of sizes. Exact figures of profit cannot be given without having costings of the various spacings, but it will be shown in the 1955 experiment that these are such as to leave little doubt that the 6×6 spacing is the most profitable in this experiment.

1954 EXPERIMENT.

Four square and four triangular spacings were included, the sides of the square or triangle being 6, 7, 8 and 9 inches. In practice the triangular spacing was obtained

Table III.

Effect of spacing on grade and yield of lettuce ; 1954 crop.

Spacing (inches)	Number planted	Numbers				Weight (kg.)				Marketable weight (tons per acre)
		in grade			Total	in grade			Total	
		18	24	30		18	24	30		
Square										
6×6	544	0	125	417	542	—	20·1	54·6	74·7	23·6
7×7	392	0	358	34	392	—	61·8	4·2	66·0	21·2
8×8	312	0	312	0	312	—	60·8	—	60·8	18·8
9×9	240	240	0	0	240	51·9	—	—	51·9	16·5
										S.E., ±0·63
Triangular										
6×5	680	0	45	635	680	—	7·0	78·8	85·8	26·0
7×6	480	0	368	112	480	—	59·5	14·5	74·0	22·7
8×7	364	0	364	0	364	—	68·6	—	68·6	20·8
9×8	288	287	0	0	287	59·8	—	—	59·8	17·8
										S.E., ±0·63

by planting the lettuces X inches apart in the row, the rows being X–1 inches apart, and by staggering the lettuces in adjacent rows. This gave an approximately equilateral triangle. A triangular spacing may be expected to make more efficient use of the ground than a square one with the same area per plant. The rectangular spacing was omitted because of the previous year's results. The plot size was 50 square feet, including the guard rows.

Results.

Table III shows the number of seedlings planted at each spacing, the number and weight, in grades, of lettuces cut at maturity, and the total marketable weight converted to tons per acre of growing area. The complete crop was harvested in one day, 12th April. Losses were again very slight, only 3 in 3,300, and the entire crop was marketed.

These figures confirm the results of the previous experiment in that each set of means shows a steady decrease in marketable weight as the area occupied per plant increases and the closest spacing yet used, i.e. 6 inch triangular, shows no sign of any falling off in this yield increase.

The yields, as shown in Fig. 1, suggest that the triangular spacing has given a higher yield per unit area for a given spacing per plant than the square spacing. A test of this has been made in the following way. It has been assumed that the two response curves of yield on area per plant can be represented as parallel quadratics, i.e. if x represents the area per plant, y the yield per unit area of a square spacing, and z the yield per unit area of a triangular spacing then

$$Y = m_1 + bx + cx^2$$
$$Z = m_2 + bx + cx^2$$

where Y=expected value of y and Z of z. Measuring x in sq. in., and y and z in tons/acre, and fitting the constants m_1, m_2, b, and c by least squares, we obtain $m_1 = 33{\cdot}32$, $m_2 = 33{\cdot}85$, $b = -0{\cdot}310$, and $c = 0{\cdot}00126$. The residual mean square after fitting is less than the error mean square of the experiment, showing that the fit is satisfactory. The estimated difference $m_2 - m_1 = 0{\cdot}53 \pm 0{\cdot}32$ tons/acre. Thus the difference does not reach significance in this experiment ; it is small in absolute value, representing an increase of 2·2 per cent. in yield at the 6×6 inch spacing.

The mean prices per crate of the three grades and the estimated gross returns per acre, as calculated from the market returns, are shown in Table IV.

Price variations between grades were rather larger in 1954 than in 1953. It will be noted that the returns for the 6×6 inch spacing are anomalous in that they do not

Table IV.

Market returns and estimated gross returns per acre ; 1954 crop.

Mean price (shillings)	Grade			Estimated gross return per acre			
	18	24	30	Spacing	£	Spacing	£
Per crate ..	14·7	17·1	15·1	6×6	4,580	6×5	5,110
				7×7	4,420	7×6	4,900
				8×8	3,490	8×7	3,990
Per lettuce	0·82	0·71	0·47	9×9	3,170	9×8	3,550

lie between those for the 7×6 and 6×5 inch spacings. This is caused by the (necessarily) large steps between the grades and the consequent grouping errors in the price determinations. Ideally the prices should be determined for each treatment separately but this has not been possible in these experiments.

1955 EXPERIMENT.

The same spacings were used as in 1954 except that the 9-inch square spacing was omitted because of an increase in plot size. The effect of spacing on crop yields had been clearly shown in the two previous experiments and hence in this one more emphasis was placed on assessing costs, where these varied with the spacing. To this end, the plot size was increased to 100 square feet including the guard rows. The plots were made as large as possible in order that the costing might be relevant to a large area, such as might be used commercially.

Results.

Table V shows the number of seedlings planted at each spacing and the number and weight, in grades, of marketable lettuces cut at maturity. The entire crop was again harvested on one day, 6th April.

Table V.

Effect of spacing on grade and yield of lettuce ; 1955 marketable crop.

Spacing (inches)	Number planted	Numbers				Weight (kg.)			
		In grade			Total	In grade			Total
		18	24	30		18	24	30	
Square									
6×6 ..	1,216	0	338	815	1,153	—	50·6	93·2	143·9
7×7 ..	896	0	432	419	851	—	65·2	49·8	115·0
8×8 ..	672	9	369	275	653	1·7	55·3	34·0	91·0
Triangular									
6×5 ..	1,428	0	189	1,106	1,295	—	27·2	121·3	148·5
7×6 ..	1,020	1	440	537	978	0·2	65·2	63·3	128·7
8×7 ..	780	11	476	274	761	2·2	73·4	32·9	108·5
9×8 ..	596	27	364	197	588	5·2	55·6	23·0	83·8

The difference between the total number planted and the total number marketed was 329 ; 32 were missing at harvest and 297 were graded as unmarketable. This increased proportion of unmarketable lettuce was due to a greater severity in grading and to a change in the strain of the variety of lettuce used. The percentage unmarketable varied from 8·8 in the closest spacing (6×5) to 0·8 in the widest (9×8).

Table VI shows marketable weight, market returns and costs for the seven spacings.

The figures for total weight have been used to estimate the efficiency of triangular relative to square spacing by the method given for 1954. The result is $m_2 - m_1 = 0 \cdot 26 \pm 0 \cdot 31$ tons/acre, about half of the 1954 value and not significantly different from zero.

TABLE VI.

Financial aspects of spacing ; 1955 crop.

Spacing	Area per plant (square inches)	Total weight (tons per acre)	Marketable weight (tons per acre)	Value per acre (£)	Labour costs per acre (excluding overheads) (£)	Value, less costs per acre (£)
6×5T ..	30	22·7	21·4	5,110	610	4,500
6×6S ..	36	20·9	20·3	4,550	510	4,040
7×6T ..	42	19·0	18·5	4,030	480	3,550
7×7S ..	49	16·6	16·2	3,450	410	3,040
8×7T ..	56	15·5	15·3	3,180	370	2,810
8×8S ..	64	13·2	13·1	2,740	320	2,420
9×8T ..	72	12·1	12·1	2,540	290	2,250

Total weight : S.E. of difference of two means, ±0·53.
Marketable weight : S.E. of difference of two means, ±0·56.

Marketable weight. These figures confirm those of previous experiments in that there is a steady increase in yield with increased plant density.

Value per acre. In calculating this the market returns have been used, together with the data of Table V. The 1955 average prices per crate of 18, 24, and 30 were 21·0, 18·1, and 15·8 shillings respectively.

Labour costs. These have been calculated from the times taken, assuming three shillings per man-hour, on most of the operations where cost varied with spacing ; they include labour for making soil-blocks, pricking-off, planting, hoeing, cutting, trimming and grading on the plots. Other costs which might vary with spacing, and which have not been included, are those for seed, packing and packing materials, glasshouse space for housing the seedlings during the propagation stages, and transport. It is thought that these would not increase the costs, as shown, by more than half at the most. It is assumed that costs relating to fuel, watering, ventilating, glasshouse depreciation and similar overheads would be constant for all spacings.

Value less costs. This figure, with the limitations described above, may be used to give the relative profit of the different spacings. The difference in profit between the extreme spacings is a matter of £2,250 per acre, which might be reduced if all the variable labour costs had been included but which would certainly exceed £2,000. Between the extreme spacings of 9 inch triangular and 6 inch triangular, i.e. from 87,000 to 174,000 plants per acre, every increase of 10,000 plants per acre means an increase in profit of about £200.

DISCUSSION.

The yields, as summarized in Fig. 1, show a tendency to vary less from year to year at the close spacings than they do at the wide spacings. It is difficult to interpret this without knowing the cause of the yearly variations in yield. Since the heating of the glasshouse was thermostatically controlled, temperature was not likely to have played a very important part. Sunshine differences suggest themselves as a possible cause, but two difficulties arise in any attempt to trace a connection between sunshine and yield. One is that the rate of growth of a lettuce crop near

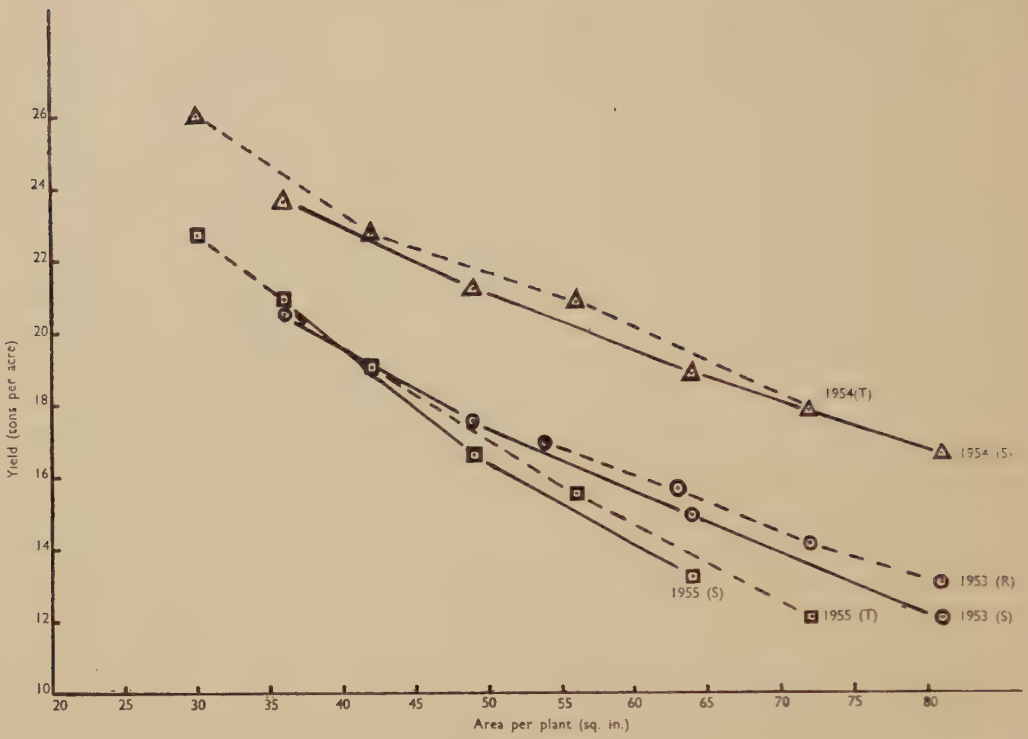

FIG. 1.

Total weight of lettuce plotted against area per plant with : (R), rectangular spacing ; (S), square spacing ; (T), triangular spacing.

maturity is extremely rapid. Smith and Nelder (unpublished) have shown that, in a cold frame, a crop may double its weight in the fortnight just prior to harvest ; thus the final yield depends very much on the cutting date. The other difficulty is that the strain of Cheshunt 5B used in 1953 and 1954 had unfortunately to be changed in 1955 and the new strain produced a few rogues, some stunted plants (probably virus-infected), and otherwise a more variable crop than the old one. Table VII gives figures for sunshine and the growing period for the 3 years.

The year 1954, which produced the largest crop, had the highest January sunshine of the 3 years and also the longest growing period. However, 1953 produced

TABLE VII.

Records of sunshine and duration of growth periods.

Year	January sunshine (hours)	January-March sunshine (hours)	No. of growing days
1953	24	207	112
1954	56	229	122
1955	33	240	116

a heavier crop than 1955, in spite of a deficiency of sunshine and a shorter growing period for the lettuce. It is possible that the difference in strains was responsible for this, but the matter cannot be taken further on these data.

A possible explanation of the lower year-to-year variation in the close spacings has been suggested to us by Dr. J. K. A. Bleasdale. It is that, at wide spacings, competition between neighbouring plants is relatively slight and the limiting factors on growth are primarily external (e.g. sunshine), whereas at close spacings competition between neighbours is relatively severe and is the dominant factor in the growth of the crop. On this basis it might be expected that variation in factors not connected with the spacing would have less effect at close than at wide spacing. The argument is hypothetical and further data would be of interest.

The shape of the lettuce is affected by spacing, close spacing producing a lettuce with a rather pear-shaped heart. Once the plants have developed to the stage of pressing against their neighbours, further growth is chiefly upwards. For this reason the cutting period of closely-planted lettuces must inevitably be short, lest the appearance of the plants suggest incipient bolting.

The three crops remained effectively free of *Botrytis* and *Bremia*, so that no observations could be made on the relative infection at different spacings. It seems possible that close spacing might encourage the rapid spread of fungus diseases, should these develop, and it may be that our results are relevant only to disease-free crops.

The combined estimate, from the 1954 and 1955 data, of the increase in yield caused by the replacement of square by triangular spacing, with the same area per plant, is $0{\cdot}39 \pm 0{\cdot}22$ tons/acre. The difference is equivalent to $1{\cdot}7$ per cent. of the mean crop at 6×6 inch spacing and does not reach statistical significance at the 5 per cent level.

We have attempted to summarize the financial aspects of spacing in Fig. 2. In this we have used the 1955 labour costs for all three years, in order to obtain a value-less-costs curve for each year. The fitted curves are fitted by least squares ; straight lines are adequate for 1953 and 1955, but a quadratic is needed for 1954. The curves can be used to read off an estimated *difference* in profit per acre of growing area for any two spacings, but not, of course, any absolute profit, since we have not attempted to evaluate overheads. We are well aware of the many limitations of these figures. For example, the market returns refer to one market at one time each year, and so the three years' results may not give a fair picture of the long-term average ; also the costs have been determined at one centre only. Nevertheless certain conclusions would remain valid for a wide range of variation in some of the variables. Thus the mean profit of the crop would still increase as the spacing declined from 9×9 inches to 6×6 inches even if the costs were doubled and the prices halved.

One other point needs to be made : there is a possibility that, in a year of glut, small lettuces from a very close spacing, say 6×5 inches, might find no sale at all, with resultant heavy loss on the crop. To this extent the returns from a very close spacing might be more variable from year to year than those from wider spacings. This is not so in the figures we have obtained, and we leave it to those with detailed knowledge of marketing conditions to assess what risks are involved in the use of very close spacings.

The crops here reported on were grown and marketed in the Midlands, a region

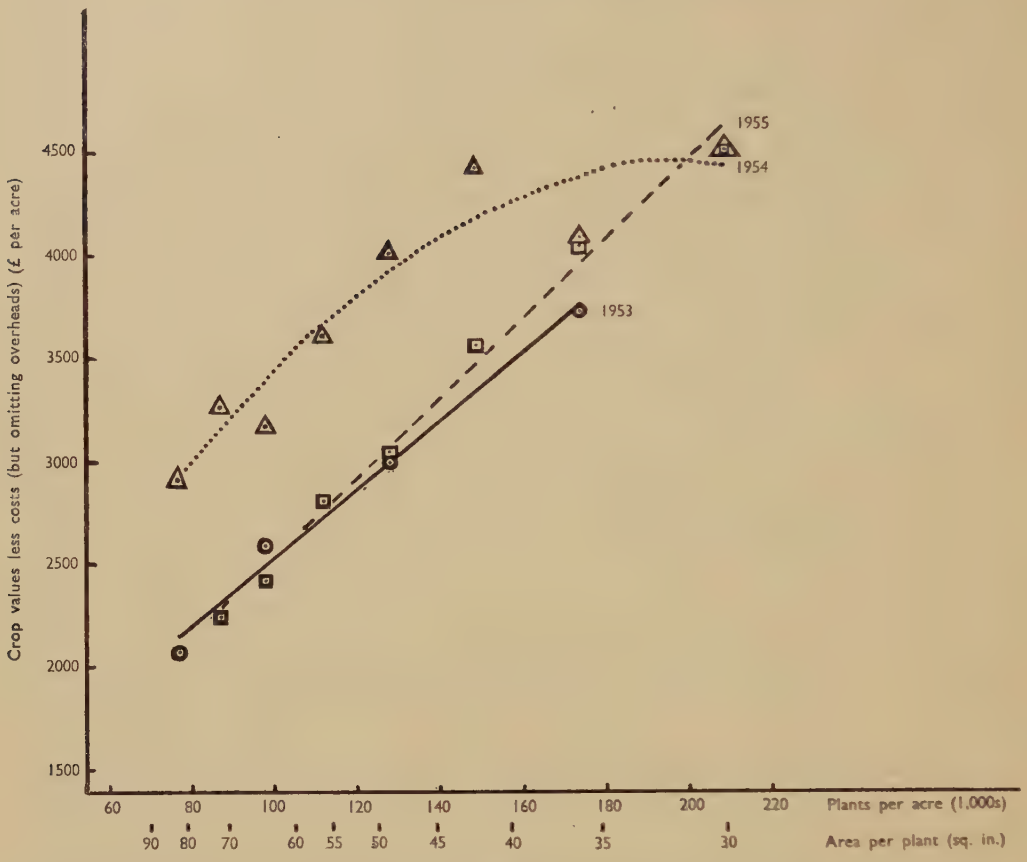

FIG. 2.

Relationship between crop value less costs (but omitting overheads) and spacing. The crop value was calculated from the market returns in each year, and the costs (omitting overheads) were estimated from 1955 data. See text for detailed explanation.

which is customarily supposed to favour a rather larger lettuce than, say, Lancashire. Nevertheless, the small lettuces from the close spacings have sold well enough in each year to give the greatest profit from the closest spacing.

SUMMARY.

1. Experiments have been carried out over 3 years at the Luddington Experimental Horticulture Station on the spacing of lettuce in heated glasshouses.

2. In all 3 years the marketable yield increased steadily as the area per plant decreased, the spacing extremes being 30 and 81 square inches per plant. Year-to-year variation was less at close than at wide spacings.

3. The value of the crop, based on actual market returns, also increased as the area per plant decreased.

4. Estimates of costs, for those operations where cost varied with spacing, were obtained from the 1955 experiment.

5. Estimates of relative profit, based on value less costs, showed that, in all 3 years, profit increased with plant density over the range of spacings tested.

6. Possible causes of year-to-year variations in yield are discussed and the limited nature of the data is stressed.

7. The mean increase in yield caused by the replacement of square spacing by triangular spacing, with the same area per plant, was 1·87 per cent. of the mean yield at a spacing of 6×6 inches, and was not significant.

ACKNOWLEDGMENT.

We are indebted to Miss Ann Meadus for drawing the text-figures and checking the calculations.

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THE VARIETAL RESISTANCE OF FRUITS TO BROWN ROT

I. INFECTION EXPERIMENTS WITH *SCLEROTINIA FRUCTIGENA* ADERH. & RUHL. ON CERTAIN DESSERT, CULINARY AND CIDER VARIETIES OF APPLE

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THE host-range of the brown rot fungi includes all the commonly cultivated fruit trees and many ornamental shrubs of the genera *Pyrus* and *Prunus* (Wormald, 1954, p. 57). Nevertheless, differences in varietal susceptibility within a species are apparent in apples, pears and stone fruits. Little is known of the causes of this variation, and the aim of this series of studies is to investigate some of the factors involved. The first paper describes experiments on the relative resistance of some representative varieties of apple fruit to infection by *Sclerotinia fructigena* Aderh. and Ruhl.

Studies on varietal resistance to brown rot in apples have been made chiefly in Austria and Germany. Thus Wahl (1926), an Austrian worker, reported that two varieties of apple (La Claimanteuse and Beauty of Boskoop), which were almost immune to *S. fructigena*, had a higher acidity and lower sugar content than several susceptible varieties, including White Astrachan. Katser (1933), on the other hand, in extensive studies of varietal resistance to brown rot, also in Austria, concluded that there was no relationship between the hydrogen-ion concentration of the fruit sap and disease resistance of different varieties. Within a given variety, however, those fruits with a higher pH value (i.e. less acid) were more resistant than those of low pH. Mittman-Maier (1940), a German worker, investigated the rate of spread of mycelium of *S. fructigena* in fruits of 158 varieties of apple following artificial inoculation. The variety Rheinischer Bohnapfel was the most resistant ; of the English varieties, Blenheim Orange and Ribston Pippin were the least susceptible.

In this country, no systematic study of brown rot resistance has been undertaken, although there are occasional references in the scientific and technical literature to the susceptibility of specific varieties. Laxton's Superb, for example, is notoriously susceptible (Anon., 1946, p. 22) and is falling into disfavour partly on this account. On the other hand, experience has shown that the disease causes virtually no losses in many cider varieties.

Preliminary observations at Long Ashton, that fruits of bitter (i.e. high-" tannin ") cider varieties were resistant to brown rot and also showed particularly rapid browning of cut surfaces, suggested that a possible relationship between these properties should be explored.

MATERIALS AND METHODS.

Apples.

The varieties used are listed in Table I, together with any previous indications of their orchard susceptibility to brown rot.

Table I.

Varieties used in brown rot infection experiments.

Name	Type	Orchard susceptibility to brown rot
Blenheim Orange	Dessert	Not high (1) (cf. Katser, 1933 and Mittman-Maier, 1940)
Bramley's Seedling	Culinary	Moderate (1)
Chisel Jersey	Cider, bitter-sweet	Low (2)
Cox's Orange Pippin	Dessert	High (Moore, 1950)
Dabinett	Cider, bitter-sweet	Low (2)
Edward VII	Culinary	Not high (1)
Ellis Bitter	Cider, bitter-sweet	High (2)
Ellison's Orange	Dessert	Not high (1)
Fair Maid of Devon	Cider, sharp	Very high (Savidge, 1947)
Frederick	Cider, sharp	High (2)
Kingston Black	Cider, bitter-sharp	Moderate (2)
Lane's Prince Albert	Culinary	Moderate to high (1)
Laxton's Superb	Dessert	High (Wormald, 1954, p. 63)
Michelin	Cider, bitter-sweet	Not known
Newton Wonder	Culinary	Not high (1)
Sweet Alford	Cider, sweet	Moderate (2)
Tardive Forestière	Cider, bitter-sweet	Not high (2)
Winston	Dessert	Not high (Roach, F. A., 1954)
Worcester Pearmain	Dessert	Moderate (1)
Yarlington Mill	Cider, bitter-sweet	Low (2)

(1) From the author's personal observations.
(2) From observations of Mr. R. R. Williams, Cider Pomologist, Long Ashton Research Station.

Inoculation.

Apples were freshly gathered from the orchard and wiped with moist cotton-wool to remove any spray deposit. As Lin (1942), in studies of fruit-rotting fungi, including *S. fructicola* (Wint.) Rehm, had demonstrated differences in the rate of mycelial growth between the " green " (shaded) and " blushed " (sunny) sides of apples, care was taken to make all inoculations on partly coloured fruits at the boundary between the green and coloured areas.

(a) *Mycelial growth.* For tests of mycelial growth rates in different varieties, a fragment of mycelium of *S. fructigena* was placed under a triangular flap of skin on each of five apples per variety. After incubation at 25° C., the mean diameter along the surface of the subsequent rot was assessed 48 and 72 hours after inoculation (unless otherwise stated), and the growth rate for the 24-hour period estimated by difference.

(b) *Infection of wounds by spores.* Two wounds were made on each of five apples per variety, by means of a special instrument (Byrde, 1952). This resembled a small cork borer, the depth of its cut being limited to 1 mm. by a flange; the disc cut out, which was 2·5 mm. in diameter, was then removed with a needle. The needle and the instrument were sterilized frequently.

The holes made were immediately filled, by means of a small pipette, with a spore suspension of *S. fructigena* (1,000 spores/ml.) in distilled water. Owing to difficulty experienced in procuring a regular supply from pure culture, spores were

obtained from artificially infected apples placed on a well-lighted shelf at laboratory temperature.

The number of wounds (out of 10 per variety) showing visible brown rot infection was assessed after 5 or 6 days' incubation at 25° C.

Test of rate of browning of fruit.

An indication of the rate of browning of the cut surface of a range of apple varieties was obtained by estimating the time taken to reach a standard colour. Pinkish cinnamon (Ridgway, 1912, Pl. XXIX) was selected after preliminary experiments.

Five apples per variety were bisected, and the number of half-apples which had reached the standard colour was assessed 1, 2, 5, 10, 20, 50 and 100 minutes after cutting. The expression $\frac{100}{t}$, where t is the time taken to reach the standard colour, was taken as an index of the browning of each replicate half-apple. The mean value for the ten replicates constituted the " browning index " of the variety at the time of the experiment.

A more refined technique, for estimating the rate of browning of apple juices, has recently been published (Weurman and Swain, 1955).

Results.

Mycelial spread.

Table II summarizes the results of experiments carried out on six occasions in 1952, with nine representative varieties of apple.

Fruits of the variety Edward VII appeared to possess a marked resistance to the spread of the pathogen. The results of further trials with this and two other

Table II.

Mean increase in diameter (mm.) of brown rot infections in the period from 48 to 72 hours after inoculation.

Variety	Date of inoculation						Overall mean
	July 25	Aug. 9	Aug. 23	Sept. 6	Sept. 20	Oct. 4	
Ellison's Orange ..	19·2	18·0	14·4	18·4	17·0	15·6	17·1
Dabinett	18·6	18·5	15·4	16·2	17·2	15·3	16·9
Fair Maid of Devon	18·9	17·7	15·4	13·6	17·4	18·1	16·9
Laxton's Superb ..	16·0	18·1	15·8	17·1	17·6	15·8	16·7
Chisel Jersey ..	19·3	14·4	15·8	16·5	16·0	16·7	16·5
Sweet Alford ..	17·8	17·7	14·6	13·9	16·2	17·8	16·3
Kingston Black ..	15·9	15·3	14·7	12·8	15·8	14·8	14·9
Bramley's Seedling	15·2	17·4	14·0	11·5	15·4	15·6	14·8
Edward VII ..	14·4	12·8	11·0	11·8	10·4	14·8	12·5
Cox's Orange Pippin	Not tested	Not tested	14·6	16·3	20·4	18·2	(17·4)
Yarlington Mill ..	Not tested	Not tested	Not tested	15·0	12·4	15·2	(14·2)

Least significant difference between overall means : P=0·05, 1·3 ; P=0·01, 1·7 ; P=0·001, 2·2. (Not applicable to data for Cox's Orange Pippin and Yarlington Mill).

varieties, presented in Table III, suggest that this feature is inherent, and not merely a peculiarity of the variety in the 1952 season.

TABLE III.

Mean increase in diameter (mm. per day) of brown rot infections in four seasons.

Variety	Date of inoculation				Overall mean
	9.8.52 (a)	8.8.53 (b)	12.8.54 (b)	25.8.55 (b)	
Laxton's Superb ..	18·1	21·4	22·3	20·2	20·5
Bramley's Seedling ..	17·4	15·7	18·0	18·2	17·3
Edward VII	12·8	14·8	15·6	13·4	14·2

Least significant difference between overall means : P=0·001, 2·5.
(a) rate estimated over 2- to 3-day period.
(b) rate estimated over 2- to 4-day period.

Infection by spores.

Table IV shows the results of spore infection experiments carried out on five occasions in 1952. For statistical analysis of the data, excluding those for Cox and Yarlington Mill, an angular transformation was applied (Anscombe, 1948).

TABLE IV.

Number of brown rot infections following spore inoculation of ten wounds.

Variety	Date of inoculation					Total	Mean per-centage	Mean of trans-formed data
	Aug. 2	Aug. 30	Sept. 13	Oct. 1	Oct. 13			
Laxton's Superb ..	10	7	5	8	10	40	80	69·0
Sweet Alford ..	8	6	4	10	6	34	68	58·8
Ellison's Orange	7	6	7	8	8	36	72	58·2
Edward VII ..	10	2	5	4	9	30	60	54·5
Fair Maid of Devon	9	3	2	8	9	31	62	53·3
Bramley's Seedling	7	4	6	8	5	30	60	51·0
Kingston Black ..	5	4	3	6	3	21	42	40·3
Chisel Jersey ..	2	0	2	4	7	15	30	29·8
Dabinett	1	0	1	4	2	8	16	20·5
Cox's Orange Pippin	Not tested	7	4	8	10	(29)	73	—
Yarlington Mill ..	Not tested	0	0	3	8	(11)	28	—

Least significant difference for transformed data : P=0·05, 17·4. (Not applicable to data for Cox's Orange Pippin and Yarlington Mill).

Four varieties were subjected to further test (Table V.) Although there was some year-to-year variability in the results for Dabinett and Chisel Jersey, both these varieties and Yarlington Mill were significantly more resistant to spore infection than Laxton's Superb over the four seasons taken as a whole.

TABLE V.

Number of brown rot infections (per ten wounds) in four seasons.

Variety	Date of inoculation				Mean per-centage	Mean of trans-formed data
	30.8.52	18.7.53	12.8.54	18.8.55		
Laxton's Superb ..	7	9	9	10	87·5	72·5
Dabinett	0	6	0	6	30·0	25·4
Chisel Jersey	0	0	0	5	12·5	11·3
Yarlington Mill ..	0	1	0	3	10·0	12·9

Least significant difference for transformed data : P=0·05, 19·1.

Correlation of spore infection with browning rate.

Study of the data of Table IV shows that "bitter" cider varieties (the last three) were resistant to spore infection, and it was observed that the cut surface of wounds on these varieties browned particularly quickly. An experiment was therefore carried out on 18th August 1955 to examine further the possibility of a relationship between rate of browning of wounds and their resistance to spore infection.

Ten fruits of each of the 20 varieties listed in Table I were collected ; five were used for a spore infection test, and five for a rate of browning test according to the method already described.

Fig. 1 shows the results obtained ; a highly significant negative correlation exists between spore infection of wounds and their rate of browning.

DISCUSSION.

The susceptibility of an apple variety to brown rot infection is clearly governed by a number of independent factors, both physical and chemical. Thus, while Horne (1933) demonstrated that, under certain experimental conditions, *S. fructigena* is able to infect an apple through a lenticel without any previous injury, the point of entry of the fungus under orchard conditions can almost invariably be traced to a wound in the cuticle (Wormald, 1954, p. 61). It follows, therefore, that the liability of an apple variety to skin injury, itself depending on other factors such as skin thickness, will affect its susceptibility to brown rot in the field (cf. Willaman, Pervier and Triebold, 1925).

The present studies, however, pre-supposed the existence of an injury and were concerned with factors which operate subsequently. The data presented indicate the existence of two distinct forms of inherent brown rot resistance in apples — resistance to the mycelial spread of an established infection, and resistance of injured tissue to the initial infection by spores.

Resistance to mycelial spread of *S. fructigena* was shown by the variety Edward VII, and to a less extent by Bramley's Seedling and Kingston Black. Edward VII, it may be noted, is a seedling of Blenheim Orange (Robb-Smith, 1955), also found to be resistant when tested in 1955. Such resistance to the spread of the pathogen appears to be akin to that described by Mittman-Maier (1940). The possibility that it could arise from the inhibition of fungal pectolytic enzymes by a fruit constituent

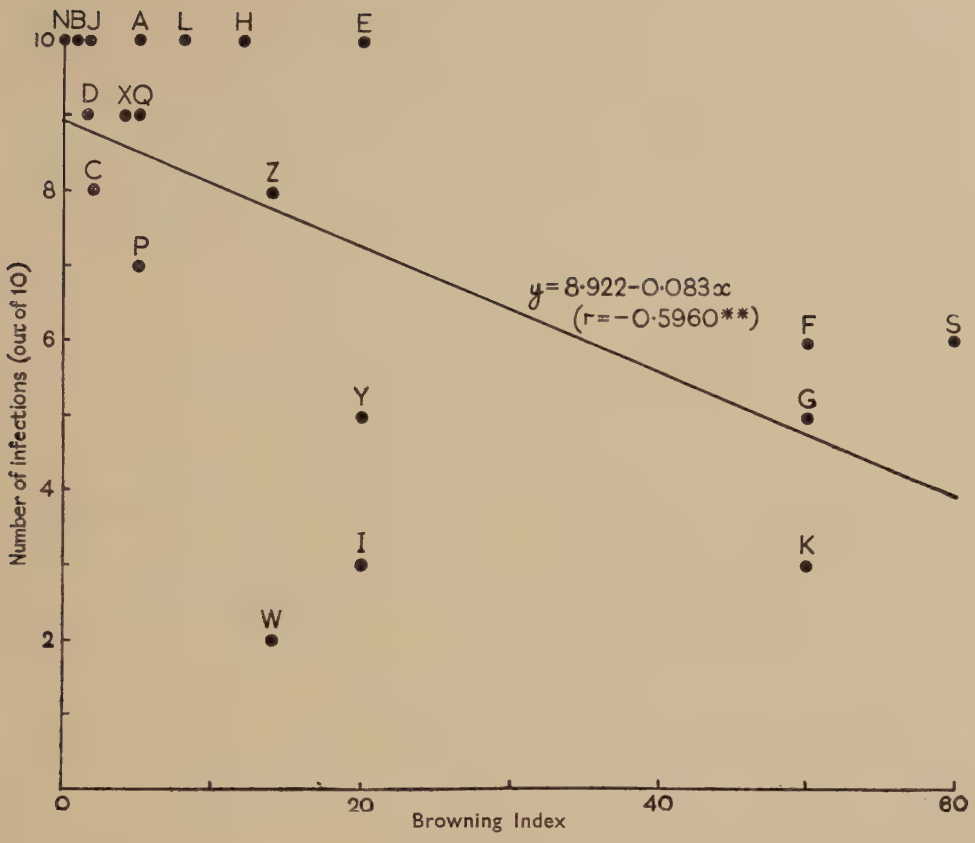

Fig. 1.

Spore infection of wounds by brown rot as related to rate of browning of cut surface. Key to varieties : A, Laxton's Superb ; B, Ellison's Orange ; C, Bramley's Seedling ; D, Edward VII ; E, Sweet Alford ; F, Dabinett ; G, Chisel Jersey ; H, Fair Maid of Devon ; I, Kingston Black ; J, Cox's Orange Pippin ; K, Yarlington Mill ; L, Winston ; N, Worcester Pearmain ; P, Blenheim Orange ; Q, Newton Wonder ; S, Michelin ; W, Tardive Forestière ; X, Frederick ; Y, Ellis Bitter ; Z, Lane's Prince Albert.

** Significant at P=0·01.

is suggested by the work of Cole (1956), who demonstrated the presence of a pectinase inhibitor in Bramley's Seedling apples, and of Weurman (1953), who reported the presence of a pectinase inhibitor in pears.

The ability to resist spore infection of a wound was found in a number of cider varieties, particularly Yarlington Mill, Chisel Jersey and Dabinett, and probably accounts for their low susceptibility to brown rot in the orchard. Moreover, a significant correlation was found to exist between resistance to spore infection and the rate of browning of injured tissue. The mechanism of such browning is not fully understood, although it is known to result from the oxidation by polyphenol oxidase enzymes of naturally occurring phenolic substances, of which chlorogenic acid has been shown to be one (Weurman and Swain, 1953). Although this constitutes *prima facie* evidence of an association, direct or indirect, between brown rot resistance and phenolic content, the existence of a significant correlation between two such

variables is no proof of a causal relationship. The role of phenolic compounds in the resistance of plants to disease is still obscure, and Gäumann (1950, pp. 273–274) pointed out that only in rare instances has such a role been definitely substantiated. New biochemical methods, however, such as paper chromatography, permit the separation and identification of plant polyphenols, and the examination of specific phenols may lead to a clarification of their part, if any, in disease resistance (see Kirkham, 1954 and Roach, W. A., 1955). Further studies on the mechanism of brown rot resistance are being undertaken, and it is hoped to report them in subsequent papers.

SUMMARY.

1. The resistance of eleven representative dessert, culinary and cider varieties of apple to brown rot (caused by *Sclerotinia fructigena*) was investigated, following artificial injury of the fruit skin.

2. The culinary variety Edward VII showed significant resistance to the spread of the pathogen through the fruit from an established infection.

3. The bitter-sweet cider varieties Yarlington Mill, Chisel Jersey and Dabinett showed resistance to infection of freshly made wounds by spores of the fungus.

4. In an experiment comprising twenty varieties of apple, a correlation was found to exist between resistance of wounds to spore infection and the rate of browning of the cut surface. The possible significance of this result is discussed.

ACKNOWLEDGMENTS.

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STUDIES OF FRUIT DEVELOPMENT IN RELATION TO PLANT HORMONES

III. AUXINS IN RELATION TO FRUIT MORPHOGENESIS AND FRUIT DROP IN THE BLACK CURRANT *RIBES NIGRUM**

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SYNOPSIS

Ether extracts were made of samples of black currant berries (var. Seabrook's Black) collected at weekly intervals from 18 days prior to fertilization until maturity. The extracts were shown to contain two acid auxins (a_1 and a_2), one neutral auxin (a_3), and one acid growth inhibitor (i_1), which could be separated by paper chromatography and assayed in biological tests. The changes in concentration of the three auxins throughout the season were studied in relation to seed and fruit development, fruit growth and fruit drop (" running off " and pre-harvest drop). It is suggested that the auxins are produced as by-products of either protein synthesis or hydrolysis taking place in different tissues and organs in a pre-determined order during the growth of the fruit. The rate of fruit growth showed a positive correlation with the concentrations of auxins 1 and 3, whereas fruit drop was negatively correlated with the concentration of auxin 2.

Gustafson in 1936 produced parthenocarpic fruits by treating the pistils of tomato flowers with synthetic growth-promoting substances. Later, in 1939, he found that similar substances (or auxins) occur naturally in fruits, and showed that their concentrations in ovules and developing seeds were much greater than in other parts of the fruit. From these results he concluded that auxins play an important role in the growth of fruits.

This claim has been supported by the findings of subsequent workers. Thus, Wittwer (1943) and Hatcher (1945) found that, in plants which have a normal sequence of fruit development, the ovary, prior to fertilization, contained either very small amounts of " free " auxin or none at all. After gametic union, however, relatively large amounts of auxin were extracted ; hence the suggestion that auxin was synthesized in the fertilized ovule and that it was probably this auxin which stimulated fruit growth. Such a hormonal control of fruit growth by the seeds was conclusively demonstrated by Nitsch (1950), who found that when the achenes of a strawberry were removed the growth of the fruit stopped, regardless of the stage of development. Moreover, on application of synthetic auxin in lanolin to the scars left by the achenes, such fruits could be made to resume normal growth.

Recent workers have focussed attention on the importance of the endosperm as an auxin-producing tissue. Thus, in rye grain, auxin has been located outside the embryo in the endosperm and the aleurone layer (Hatcher, 1945), and in the apple the concentration of auxin in the relatively thin layer of the endosperm was found to be many times greater than that in the embryo itself (Luckwill, 1948).

* Part of a thesis approved for the Degree of Doctor of Philosophy in the University of Bristol.

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The auxin level in the seed does not remain constant during the development of the fruit, but shows one or more fluctuations and finally a decrease as the fruit matures. Such studies were made for corn by Avery, Berger and Shalucha (1942) and by Wittwer (1943) ; for rye by Hatcher (1945) ; for apple by Luckwill (1948, 1953) ; and for strawberry by Nitsch (1950). Luckwill showed that in apple the changes in auxin content were correlated with the periodicity of fruit drop, the peaks of auxin concentration corresponding with the periods of low fruit drop.

The above investigations were carried out with crude extracts which undoubtedly contained both auxins and growth-inhibitors. Larsen (1939) showed that ether extracts of green tomato fruits contained at least two growth-inhibitors. It was not until the advent of paper chromatography that a suitable technique became available whereby auxins might be separated from growth-inhibiting substances. Using this technique, Luckwill (1952) was able to show that ether extracts of asparagus fruits contained both growth-promoting and growth-inhibiting substances. The author later (1954) demonstrated that this was true also for black currant and gooseberry fruits, and the present study was undertaken to find out what part these growth-regulators might play in fruit morphogenesis and in controlling fruit drop (" running off ") in black currants.

Experimental methods.

Extraction and fractionation of growth-regulators.

Random samples of 2,000 berries were picked from field-grown bushes at weekly intervals from 18 days prior to fertilization until maturity. Each bush was sampled only once, the berries being picked from the first three positions on each truss. The berries were frozen at —20° C. for 48 hours, ground to powder and extracted with ether (A.R. grade) at 2° C. for 21 hours. During this period the ether was changed twice, for each change 5 ml. of ether being used per gm. fresh weight of berries. The extracts were bulked, the ether distilled off at 75° C., and the residue digested in distilled water at the same temperature for 5 minutes, using 1 ml. per 5 gm. fresh weight of berries. The extract was left at 2° C. for 24 hours, after which the solidified fatty layer containing chlorophyll was filtered off. The filtrate was extracted three times with three times its own volume of ether. The first ether extract was shaken with one-sixth of its volume of saturated sodium bicarbonate solution. The bicarbonate solution was drained into the second ether extract, shaken, and then into the third. Each ether extract was shaken with eight separate volumes of bicarbonate solution by this counter-current extraction method.

The three ether extracts, containing the non-acidic (neutral) substances, were bulked, dehydrated with anhydrous sodium sulphate and taken to dryness in a small test-tube by drip distillation. The bulked bicarbonate solution, containing the acidic substances, was acidified to pH 3 with 20 per cent. tartaric acid and shaken with three times its volume of ether, using three changes of ether. The ether was bulked, dehydrated and distilled to dryness as above. The dry extracts were stored at —20° C. until required.

Chromatographic technique.

The dried extracts were dissolved in known volumes of absolute alcohol and spotted on to sheets of Whatman No. 1 filter paper (10¼ in. wide) with the aid of a

hypodermic syringe. The chromatograms were run, according to the descending method, in a glass tank shielded from draughts. The developing solvent was butanol-ammonia (0·88)— water (100:100:8, upper layer) and this was allowed to run to a pencil line 20·5 cm. from the starting line. After development the chromatograms were dried quickly in a draught oven at 50° C. until free of all traces of the solvent and then stored in the dark. They were tested, either biologically or chemically, within four hours of drying. Before testing they were marked out in 1 cm. or 0·5 cm. sections so as to give a total of twenty-one or forty-two sections respectively. Control sections were taken from the top of the chromatogram paper.

Detection of auxins and growth-inhibitors on the chromatogram.

Auxins and growth-inhibitors were detected by one or more of the following tests :—

Wheat coleoptile cylinder test. A coleoptile test, using wheat instead of oat, has been described briefly by Luckwill (1952), and was essentially the method employed in this study.

The sections, marked on the chromatogram as described above, were cut out and each was placed into a shallow glass dish containing 1 ml. of distilled water. Etiolated wheat coleoptiles (var. Holdfast), 2–2·5 cm. long, were lined up on a board in groups of twenty and a cutter severed them 4 mm. from the tips and cut two sections of 2 mm. from each coleoptile below this point. Ten sections, comprising five upper and five lower ones from ten different coleoptiles, were added to each dish. The coleoptile sections were incubated in a water-saturated atmosphere in a Pyrex dish at 25° C. for 24 hours. The length of each section was measured with a micrometer eyepiece. The mean length of the ten sections in each dish was then plotted against the position of the segment on the chromatogram to give a histogram as shown in Fig. 1. In these histograms the central line represents the mean length of 40 sections ; peaks to the right and left of the line indicate the location of auxins and growth-inhibitors respectively. Only the peaks above the broken lines are significant at the 5 per cent. level.*

Coleus abscission test. This is based on the ability of certain auxins to delay the abscission of de-bladed petioles of *Coleus*. The test, which has been described by Luckwill (1956), is carried out with nodal explants maintained at 25°C., the auxin being applied directly to the petioles in filter paper discs. In testing chromatograms, discs 0·5 cm. in diameter are cut out with a paper punch in sequence down the chromatogram and, after moistening with distilled water, each disc is placed on a petiole. The petioles with control discs usually drop within 24 hours, whereas those with discs containing auxin may remain attached up to 21 days.

The results are plotted as a histogram in a similar way to the wheat coleoptile cylinder test, the central line in Fig. 1 representing the mean number of days to abscission of eight control petioles.

* The following formula was used to calculate the approximate 5 per cent. fiducial limits of the control line, outside which a sample of 10 treated coleoptile sections must fall in order to be significant :—

$$\text{Approximate 5 per cent. fiducial limits} = \pm 2\,\sigma\sqrt{\frac{n+p}{np}}$$

where σ = the standard deviation of the control coleoptile sections,
n = the number of control coleoptile sections,
p = the number of treated coleoptile sections.

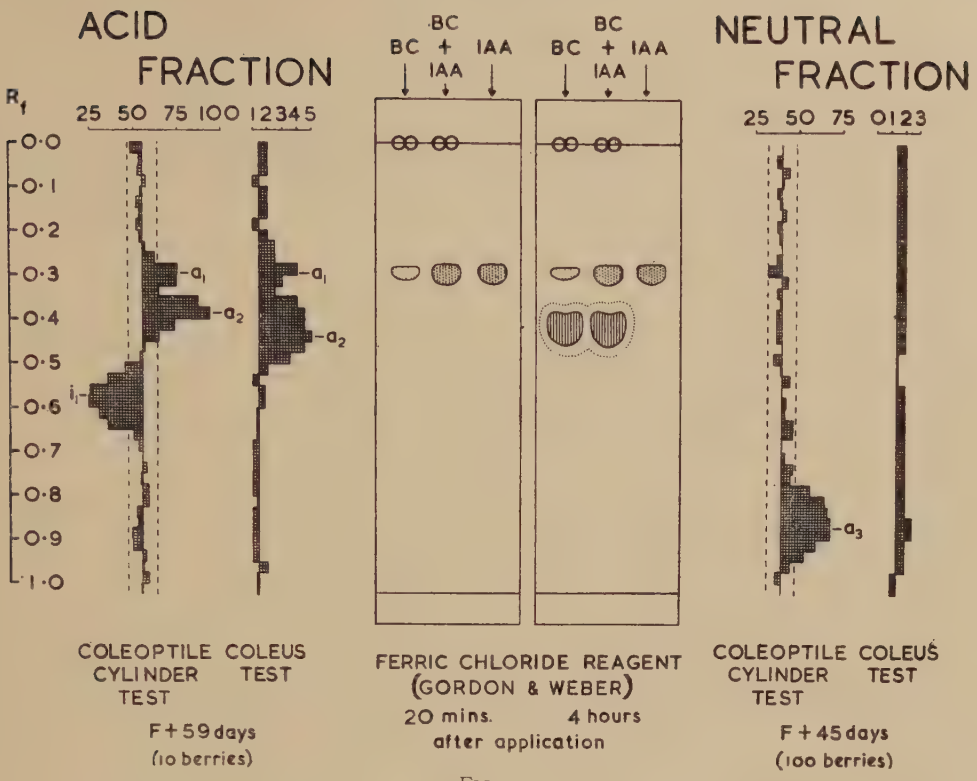

FIG. 1.

R_F values, biological activity and reaction to the ferric chloride reagent of the auxins (a_1, a_2, a_3) and the growth-inhibitor (i_1) of the black currant berry. BC is an acidic black currant fruit extract equivalent to 20 ripe berries and IAA is 3 μg. of indole-3-acetic acid.

Figures above the coleoptile cylinder tests indicate percentage increase over the original length of the sections ; those above the *Coleus* abscission test indicate the persistence of the petioles in days.

F+59 days : berries taken 59 days after fertilization ; similarly for F+45 days.

Chemical colour tests. The chromatograms were tested with a modified Salkowski reagent (Gordon and Weber, 1951) for the detection of IAA and related compounds. Ehrlich's reagent was used to detect indole-3-acetonitrile (Bennet-Clark *et al.*, 1952).

Fruit growth and fruit drop records.

Fruit growth was recorded as increase in fresh weight, dry weight and berry diameter. The fresh and dry weights were determined weekly from 500 berries collected with the main sample. After weighing, the berries were dried in an oven at 100° C. until they had reached a constant weight. Fruit diameter was obtained from the mean of 60 berries, made up of 20 from each of the first three positions on the raceme and collected from 20 bushes.

The fruit drop was recorded weekly from 15 first year shoots on separate bushes selected at random in the plantation. No samples were taken from these bushes. The total number of berries remaining on each shoot was recorded and from these

records the rate of drop was calculated by means of the exponential equation proposed by Vyvyan (1946).

Histological methods.

Berries from the second position on the raceme were fixed and embedded in paraffin wax. Serial sections of 10μ thickness were taken both transversely and longitudinally through the centre of the berries. The sections were double-stained with Delafield's haematoxylin and safranin.

Characteristics of the growth-regulators in the developing fruit.

Black currant fruits were found to contain three auxins and one growth-inhibitor which, until they have been identified chemically, may be called *Ribes* auxin 1, *Ribes* auxin 2, *Ribes* auxin 3 and *Ribes* inhibitor 1.* Dosage/response curves were constructed for auxins 1 and 2 and for inhibitor 1 by eluting many chromatograms at the correct R_F range, concentrating the extract and diluting to a range of concentrations and testing these in the selected biological test.

Auxin 1 is an acidic substance with the same R_F value (0·3) in butanol/ammonia as indole-3-acetic acid (IAA). It is active both in the coleoptile cylinder test and in the *Coleus* abscission test, and like IAA gives a pink spot with the modified Salkowski reagent. However, when the dosage/response curves of IAA and auxin 1 in the wheat coleoptile cylinder test are compared (Fig. 2c), it is clear that auxin 1 has a lower maximum activity and is active over a more limited range of concentrations than IAA. This sample of auxin 1 was collected by elution of a number of separate chromatograms between the R_F values 0·225 and 0·325, and it is possible that the difference in the dosage/response curves noted above was due to contamination with growth-inhibiting substances which trailed on to this portion of the chromatogram. In certain tests in which this auxin was eluted from a narrower zone of the chromatogram, an activity of the same order as that induced by the optimum concentration of IAA was found. Hence, despite the difference in the response curves, it seems likely that auxin 1 is identical with IAA.

Auxin 2 is an acidic auxin with an R_F value of 0·42 in butanol/ammonia (i.e. approximately 15 per cent. higher than that of IAA). It is active in both biological tests and gives a pink spot with the modified Salkowski reagent after a period of four hours (Fig. 1), suggesting that it is gradually converted into IAA or some related compound in the presence of the reagent. The discrepancy in the R_F value of auxin 2 in the two tests (Fig. 1) is due to the trailing of the growth-inhibitor which, being active in the wheat coleoptile cylinder test, eliminated the lower portion of the auxin 2 peak in this test.

In Fig. 2, a and b, dosage/response curves for IAA are compared with similar curves for auxin 2. It will be noticed that, for the lower part of the range of concentrations used, the magnitude of the coleoptile cylinder response in relation to that of the *Coleus* response is greater for IAA than for auxin 2.

It is possible that auxin 2 might be a precursor of auxin 1. If this were so, one would expect the changes in concentrations of auxin 2 during the development of the berry to be related to those of auxin 1. However, as will be described later, this is not so, and one can only conclude that auxin 2 is an independent, though chemically

* In the text which follows, the name *Ribes* will be omitted.

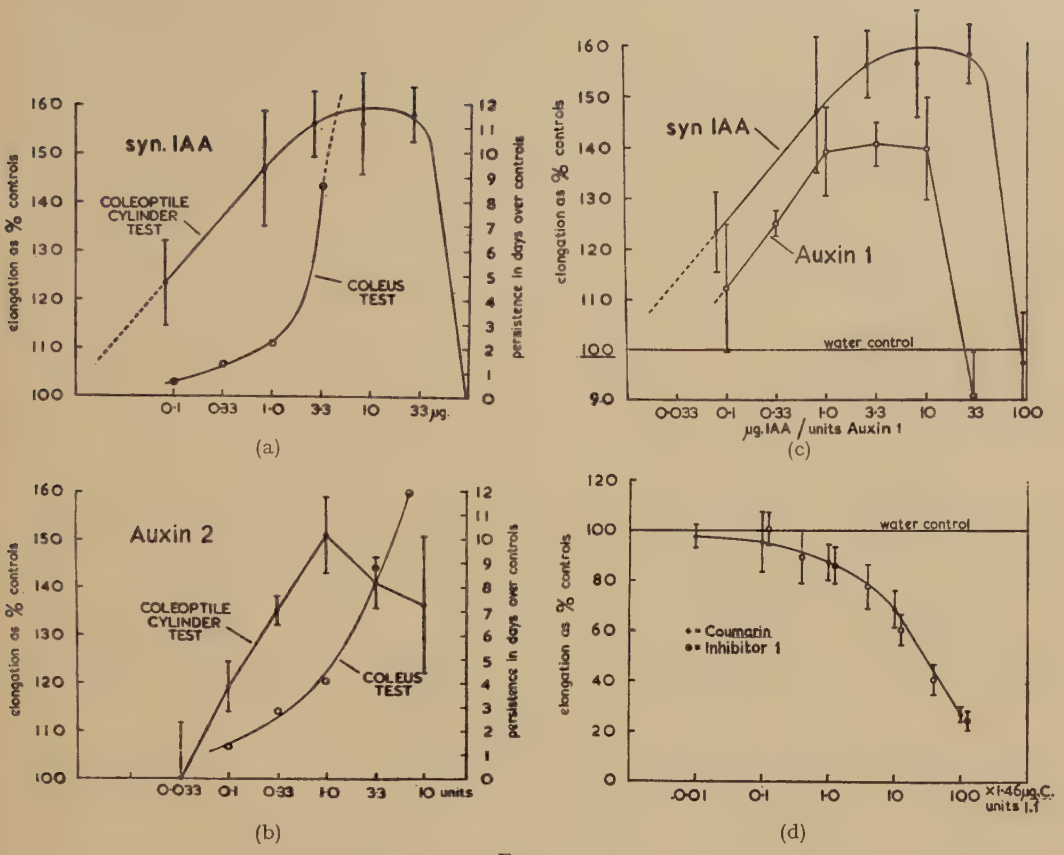

FIG. 2.

Dosage-response curves of black currant auxins and synthetic growth-regulators compared.
a. Synthetic IAA in the coleoptile cylinder test and the *Coleus* abscission test.
b. Auxin 2 in the coleoptile cylinder test and the *Coleus* abscission test.
c. IAA and auxin 1 in the coleoptile cylinder test.
d. Coumarin and inhibitor 1 in the coleoptile cylinder test.
The vertical lines through the points represent ±2 S.E.

related, auxin. It appears not to be identifiable with any of the indole derivatives tested by Stowe and Thimann (1953) or by Sen and Leopold (1954).

Auxin 3 is a non-acidic auxin with an R_F value in butanol/ammonia of 0·87. It is active in the wheat coleoptile cylinder test, but only very slightly active in the *Coleus* abscission test. It gave no colour reaction with the modified Salkowski reagent but with Ehrlich's reagent there was a faint blue-coloured spot, which occurred at the same R_F value as a control spot of indole-3-acetonitrile. Luckwill (1956) has recently found that IAN is only slightly active in the *Coleus* abscission test and this evidence, together with that of the R_F value, suggests that auxin 3 might be identical with IAN.

Inhibitor 1 is an acidic growth-inhibiting substance with an R_F value of 0·57 (Fig. 1). It is active in the wheat coleoptile cylinder test, its presence being detected

by reduced elongation of the coleoptile sections compared with the controls, but it is not active in the *Coleus* abscission test. It gives no colour reaction with either modified Salkowski or Ehrlich's reagent.

The dosage/response curve is almost identical with that of coumarin and the two curves are shown superimposed in Fig. 2d. The two substances are not the same, since inhibitor 1 is an acidic and not a neutral substance. Nevertheless, the close similarity in activity over a considerable range of dilution suggests that they may be related chemically. Unlike coumarin and its derivatives, however, inhibitor 1 shows no ultra-violet fluorescence at alkaline pH values, even when present in amounts equivalent in biological activity to 100 μg. coumarin.

Quantitative changes in auxins and growth-inhibitor during fruit development.

Bioassay method.

Each auxin and the growth-inhibitor were estimated quantitatively by converting the growth response of each section of three replicate chromatograms in the relevant R_F zone into equivalent units from the respective dosage/response curve. By addition of the values obtained, the total quantity of the substance present on the chromatogram was estimated. Trial chromatograms of the acidic and neutral fractions of each sample were first tested biologically to ascertain for each growth-regulator a suitable concentration of the extract to be applied to the chromatograms to be bioassayed. This was necessary to ensure that the amounts of the growth-regulator on the chromatograms would give a growth response which fell within the sensitive part of the respective dosage/response curve.

Auxins 1 and 3 were bioassayed in the wheat coleoptile cylinder test and expressed as μg. equivalents of IAA. To avoid the masking effect caused by the trailing of the inhibitor, auxin 2 was bioassayed in the *Coleus* abscission test. Since the slope of the dosage/response curve of this auxin was different from that of IAA in this biological test, auxin 2 was measured in arbitrary units. Inhibitor 1 also was expressed in arbitrary units by using the dosage/response curve shown in Fig. 2d.

Changes in content of auxins and growth-inhibitor during fruit development.

Changes in the total amounts of the growth-regulators in the fruit during development are shown for auxins 1 and 3 in Fig. 3, for auxin 2 in Fig. 4a, and for inhibitor 1 in Fig. 4b.

Auxin 2 was first detected soon after fertilization, rose steadily to a peak between 45 and 52 days, and then decreased as the berries ripened.

Auxins 1 and 3 both showed two peak periods. The first was about 24 days after fertilization and the second about 45–52 days. As with auxin 2, activity decreased rapidly before harvest. The similarity between the fluctuations of auxin 1 and auxin 3 suggests that one might be the precursor of the other. During 1952, auxin 3 was the only auxin estimated quantitatively, and a similar double peaked curve was again obtained.

Inhibitor 1 increased in quantity throughout the growth of the fruit, following fairly closely the increase in dry weight. There was no decrease of the inhibitor prior to harvest.

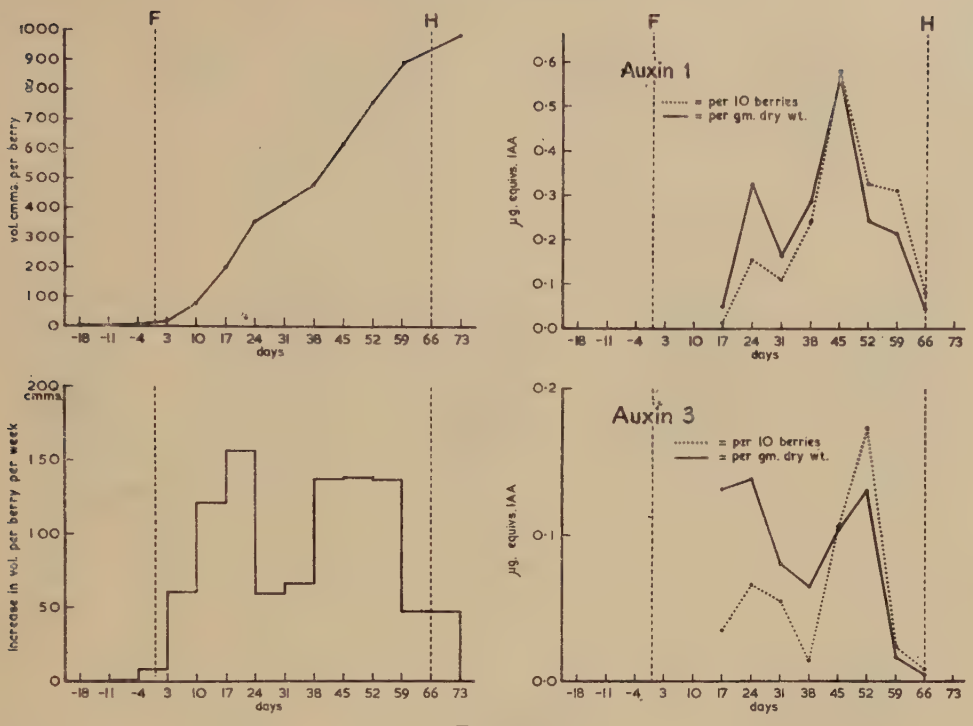

FIG. 3.

Berry growth and auxin content.
F, time of fertilization ; H, harvest time.

SEED AND FRUIT DEVELOPMENT.

The histological development of the seed and fruit is shown diagrammatically in Fig. 5.

Prior to fertilization the ovule grows slowly, mainly by cell division. After fertilization the growth of the berry can be divided into three main phases :—

1st phase (*0–24 days after fertilization*). This is characterized by the rapid swelling of the berry as a result of cell enlargement taking place within the tissues of the pericarp. A cork cambium develops in the apical hypoderm and proceeds to cut off many layers of cells to protect the upper part of the berry as the flower dies.

There is a rapid increase in the size of the seeds. The nucellus cells continue to divide, particularly at the chalazal end. The triple-fusion nucleus undergoes many divisions, filling the embryo-sac with many free endosperm nuclei before cytokinesis occurs. Many of the cells thus formed divide frequently throughout the remainder of this phase, whilst other cells begin to enlarge. The embryo grows very slowly.

2nd phase (*25–38 days after fertilization*). The volume of the berry increases slowly, but the seeds grow rapidly. The endosperm increases in size, breaking down and absorbing the nucellus ; a few of the endosperm cells continue to divide for a

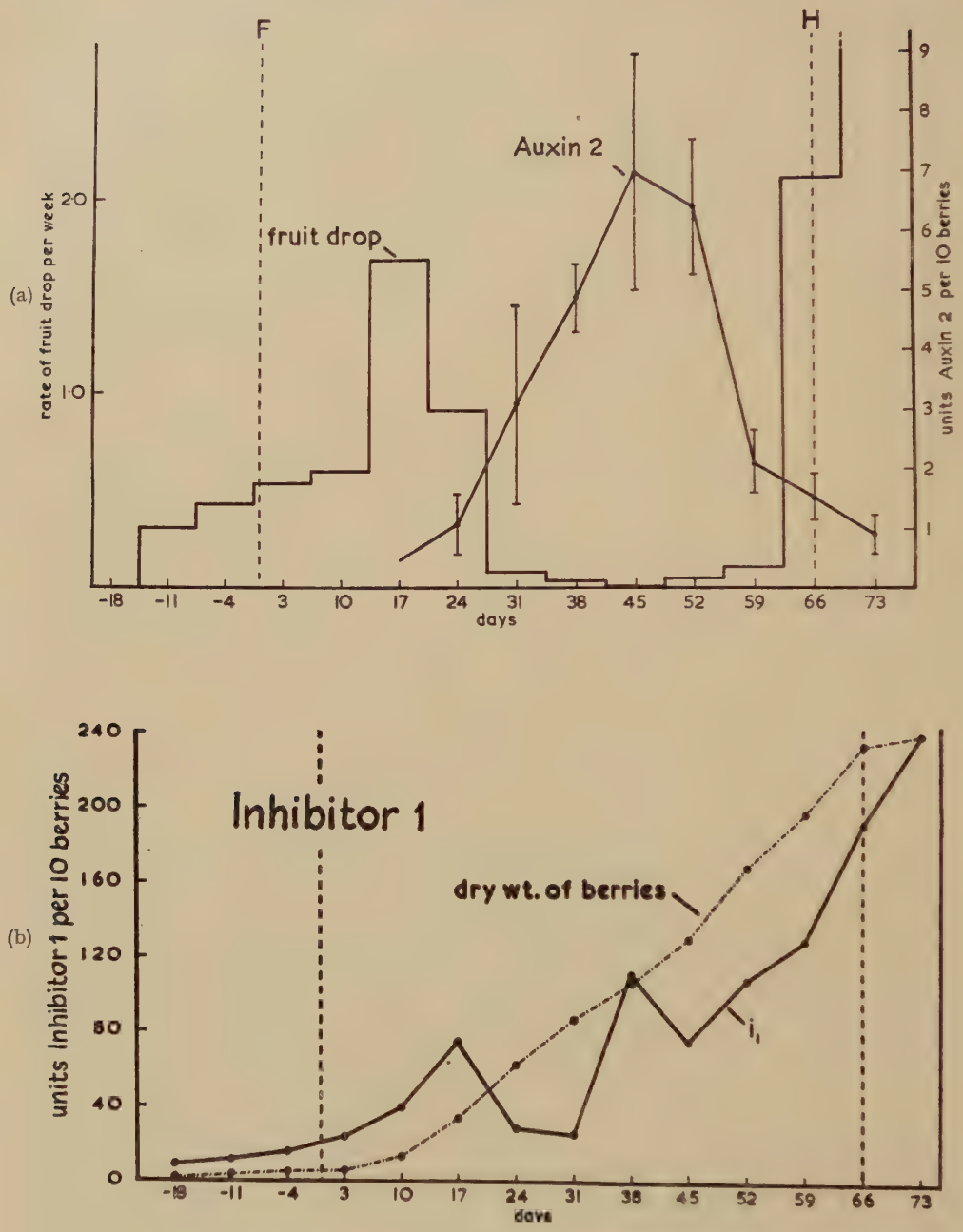

FIG. 4.

a. Berry drop and auxin 2 content during development.
Vertical lines through the Auxin 2 points represent ±2 S.E. F, H, as in Fig. 3.
b. Fluctuations in content of inhibitor 1 during development of the black currant fruit (full line).
Increase in dry weight of berries (dotted line).

PLATE I

Fig. 5.

Seed and fruit development of black currant (var. Seabrook's Black).

Above : longitudinal sections of seeds (×20) during development : t., large cell layer of testa derived from outer integument ; w., portion of testa derived from inner integument ; v.b., vascular bundle ; n., nucellus ; en., endosperm ; d.en., digested endosperm ; and em., embryo.

Below : transverse sections of berries (×3) during development.

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while, the rest enlarge and many contain granular inclusions due to the building up of food reserves. The embryo grows slowly.

3rd phase (39–73 days after fertilization). The berry volume increases rapidly, largely because of cell enlargement taking place in the placentae. The outer tissues of the pericarp become stretched and pigmented.

The seeds increase in size for a short while. The endosperm cells enlarge and simultaneously accumulate protein reserves which are present as numerous aleurone grains in the mature cell. The embryo undergoes rapid cell division and differentiates into cotyledons, plumule and radicle. When fully grown the embryo occupies only a small part of the endosperm.

Correlation of results.

Fruit growth and auxin content.

The berries showed two peak periods of rapid growth (Fig. 3). Histological study showed that the first of these was due primarily to cell enlargement in the pericarp and the second to cell enlargement in the placentae. The reason for these two cycles of cell enlargement may be that the placental cells remain meristematic much longer than do those of the pericarp. Since fruit growth after fertilization is attributable chiefly to cell enlargement, and since auxins have the ability to bring about cell enlargement, it is of particular interest to note that auxins 1 and 3 also show two peak periods during the development of the berry.

In Fig. 3 the two sets of results are shown for comparison. It is seen that the berries increase rapidly in volume up to the 24th day after fertilization ; similarly the amounts of auxins 1 and 3 increase up to the 24th day. The volume of the berry again begins to increase rapidly at the 38th day ; the amounts of auxins 1 and 3 also begin to increase rapidly at this stage. At the 66th day the rate of increase in volume is decreasing rapidly ; at the 66th day both auxins have fallen to very low levels. Thus in the black currant berry there is a correlation between the concentrations of auxins 1 and 3 and the rate of fruit growth.

Auxin 2 might also play a part in fruit growth. If this is so, it is rather puzzling that there is an intermediate period of relatively slow growth seeing that, during this period, auxin 2 is present in appreciable amounts. On the other hand, this auxin may have been responsible for the rapid enlargement of the endosperm cells during this phase.

Protein synthesis and auxin production.

It is well known that IAA and related auxins or their precursors are formed in regions of active protein synthesis (e.g. meristematic tissues). By studying the histological results it was hoped that it might be possible to deduce where the various auxins were being synthesized.

During the development of the black currant berry, two types of protein synthesis were found to be taking place concurrently, one associated with the formation of new protoplasm, the other with the accumulation of protein reserves in the endosperm in the form of aleurone grains.

From Fig. 6 it will be seen that the first peaks of auxins 1 and 3 could result from meristematic activity in the endosperm. However, it is possible that these auxins are formed in more than one tissue, in which case the peaks could be a combination

of auxin derived from all tissues meristematic at this time. The latter would include the cork cambium in the hypoderm, and testa, embryo and endosperm of each seed. Of all these tissues the endosperm was the most active as regards the rate and numbers of cell divisions taking place. The second peak of auxins 1 and 3 could be associated with the rapid cell divisions within the embryo.

Auxin 2 did not appear to be correlated with meristematic activity within the fruit, since it increased steadily from about 10 until about 48 days after fertilization. It is suggested, however, that it might be produced as a by-product of the second type of protein synthesis taking place within the fruit, namely the accumulation of reserve protein within the endosperm, which starts within a few days of fertilization and continues until the seed is fully grown (Fig. 6).

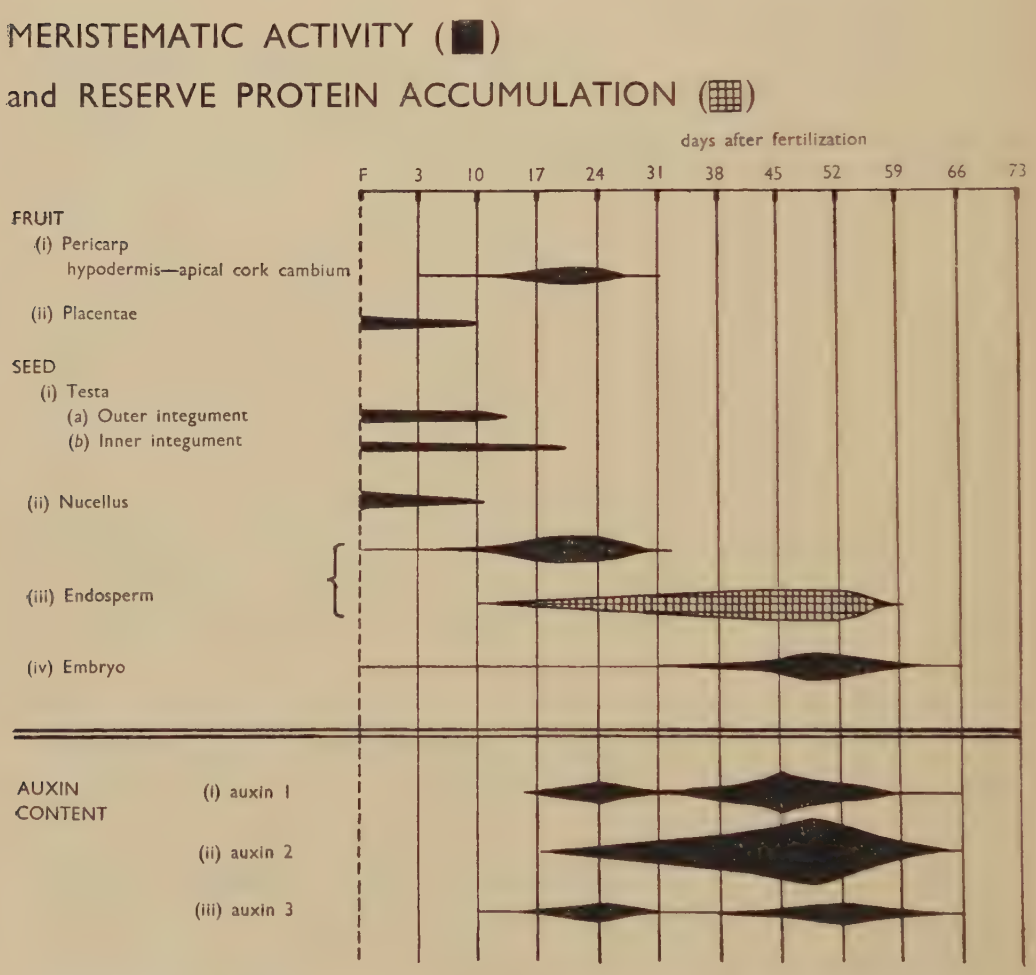

FIG. 6.

Diagram showing the changes in auxin content of the black currant in relation to meristematic activity and reserve protein accumulation in the different tissues of the fruit.

Fruit drop and auxin content.

One of the objectives of this study was to find whether the early drop of berries, known to growers as " running off ", was correlated with auxin content. From the fruit drop histogram in Fig. 4a it will be noticed that the early drop was spread over a period of about six weeks. The parts dropped included abnormally developed flowers, unpollinated flowers as well as fertilized berries which had begun to swell. The drop which occurs when the berry is mature is not a serious concern to the grower provided picking operations are not delayed.

Since auxin 2 was active in delaying abscission of *Coleus* petioles and was present in considerable quantities, it is of interest to compare the changes in its concentration with the rate of fruit drop. From Fig. 4a it is obvious that there is a strong negative correlation between these two variables. As the amount of auxin 2 increased, so the rate of fruit drop decreased, the lowest rate of fruit drop corresponding to the presence of the maximum amount of auxin 2. As the fruit became ripe, the amount of auxin 2 decreased and the drop increased rapidly. It can be concluded therefore that both " running off " and pre-harvest drop are correlated with low auxin 2 content of the fruit.

DISCUSSION.

In this study a correlation has been established between fruit growth, due primarily to cell enlargement, and auxin content of the fruit. Of the three auxins investigated, two were found to be directly correlated with the fluctuations in rate of fruit growth : these were the acidic auxin 1 (IAA) and the non-acidic auxin 3 (IAN ?). Neither of these was detected as early as one would have expected, if they are to explain the early rapid growth rate. However, it is possible that, in the early stages of berry growth when the cells of the pericarp are small, the concentration of auxin required to bring about a certain increase in cell volume would be less than that required later when the cells are bigger. Alternatively, factors other than auxin may control the initial stages of fruit growth.

The results indicate that the intermediate period of slow growth of the fruit might be brought about by a temporary shortage of auxin. In this intermediate stage of slow growth, auxin 2 is present in appreciable quantities, but this auxin appears to be concerned chiefly with the prevention of fruit abscission. If, as this work would indicate, auxin is limiting fruit growth at this stage, the application of a synthetic auxin might be expected to increase the growth rate of the berry and lead to earlier maturity, as Blondeau and Crane (1950) have shown for the fig and Crane and Brooks (1952) for the apricot.

The fact that the content of the acid growth-inhibitor followed fairly closely the increase in the dry weight of the fruit suggests that it might be a waste product of metabolism normally locked up within certain cells, and only released by the extraction process. There is no evidence to indicate whether or not it plays any essential role in the control of growth processes within the fruit.

A correlation between certain types of protein synthesis and the presence of auxin has been suggested. If one assumes that the precursor of IAA is tryptophane, then this correlation, at least for auxin 1, was to be expected, for free tryptophane would be available in tissues where active protein synthesis is taking place. Alternatively, the tryptophane might originate from the hydrolysis of newly-formed protein for, as reported by Pearsall (1949), there is now strong evidence to suggest

that plant proteins, like those of animals, are continually being broken down and built up throughout the life of the plant cell.

However, as Gunckel and Thimann (1949) have found, enzymatic conversion of auxin precursor to free auxin does not necessarily occur in the regions of protein synthesis. For *Ginkgo* and certain monocotyledons they have shown that the seat of conversion is in the older parts of the stem and not in the apical meristem itself.

Several workers have reported that protein synthesis may continue during cell extension or enlargement (Frey-Wyssling and Blank, 1940 ; Brown, 1946 ; Thimann and Christiansen, 1950 ; and Robertson and Turner, 1951) and further that IAA is necessary before this type of protein synthesis can take place (Baldinovinos de la Pena, 1953). Robertson and Turner showed that protein synthesis in the apple persists after cell division has ceased, so that the ratio between the protein nitrogen per cell and the surface area of the cell wall remains constant. The part played by IAA in the initiation of protein synthesis in elongating cells is believed by Thimann and Christiansen to be an indirect one and they consider it probable that it exerts its effect by mediating the metabolism of the 4-carbon acids. Thus one might postulate that, in the black currant fruit, IAA is formed as a by-product of protein synthesis at the meristems and then, indirectly, controls the protein synthesis of the enlarging cells.

The early drop of fertilized berries has been shown to be inversely correlated with the content of auxin 2, which is also an indole derivative. Therefore it might be expected that those berries with the largest number of seeds would have the best chance of being retained, since they would be able to produce the greatest total quantity of auxin 2. Teaotia (1953) has confirmed that in black currant, as in other fruits, the dropped fruits have a lower average seed content than those which remain attached. Their lower seed content is due, however, not to failure of fertilization, but to seed abortion, which suggests a nutritional interpretation of fruit drop. This seems to be supported by the fact that the drop increased towards the apex of the raceme, where presumably less nutrients would be available.

In order to link the auxin theory of fruit drop with that of a nutritional interpretation, the following hypothesis is put forward :

It is suggested that the auxins required for fruit growth and for the prevention of abscission are formed as a consequence of protein synthesis at meristems and of the synthesis of reserve protein within the endosperm. During the growth of the black currant berry it was found that these types of protein synthesis take place, in different tissues and organs, in a predetermined order. Thus the responsibility of producing auxin passes from one tissue to the next during the growth of the fruit.

According to this viewpoint the growth and retention of the fruit would be dependent on the maintenance of active centres of protein synthesis. Moreover, since the retention of the fruit apparently depends on the diffusion of a certain critical amount of auxin, particularly auxin 2, from the fruit, then presumably the rate of protein synthesis in the endosperm is also an important factor.

Now, if food materials were limited, there would be competition between the berries to obtain sufficient for their own requirements. In accordance with their position and vascular supply, some berries would obtain sufficient nutrients to maintain protein synthesis at the rate necessary to ensure the production of enough auxin to prevent abscission, while in other berries the rate of protein synthesis in the critical tissues and organs would be insufficient and abscission would follow. Thus " running

off " could be caused by a shortage of available food materials, which indirectly brings about a fall in auxin content in some berries and so leads to berry drop.

Likewise, the drop of unfertilized ovaries could be explained by the above hypothesis, for in these the important centres of protein synthesis associated with auxin production (i.e. the endosperm and the embryo) are absent altogether.

SUMMARY.

1. Ether extracts of black currant berries were made at weekly intervals from 18 days prior to fertilization until maturity.

2. The auxins and growth-inhibitors in the extracts were separated by the technique of paper chromatography and detected and assayed by one of two biological tests, (i) a modified coleoptile cylinder test and (ii) a *Coleus* abscission test. Certain of the auxins were also detected by colour reagents (Gordon and Weber's reagent and Ehrlich's reagent).

3. At various stages throughout the growth of the fruit one neutral auxin and two acidic auxins were found, together with one acidic growth-inhibitor.

4. One of the acidic auxins and the neutral auxin show many characteristics in common with IAA and IAN respectively. The other acidic auxin also is probably an indole derivative, as it gives a delayed colour reaction with Gordon and Weber's reagent.

5. Variations in the amounts of the three auxins and of the growth-inhibitor in the berry were followed throughout the season.

6. Meristematic activity within the berry and the accumulation of reserve protein in the endosperm of the seed were found to coincide with the auxin peaks.

7. The growth of the black currant fruit was correlated with the presence of one of the acidic auxins (IAA) and of the neutral auxin (IAN ?).

8. The peak concentration of the other acidic auxin was associated with a period of low fruit drop.

9. A working hypothesis is suggested to account for the interaction of hormonal and nutritional factors in the control of fruit drop.

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BACTERIAL CANKER OF STONE-FRUITS

II. LEAF SCAR INFECTION OF CHERRY

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In Bacterial Canker of cherry, caused by *Pseudomonas mors-prunorum* Wormald, a summer leaf spot stage alternates with a winter canker stage on the stems and branches (Wormald, 1937 ; Crosse, 1954). Recent field observations of natural infections (Crosse, 1955) lent considerable support to the view, originally expressed by Montgomery and Moore (1945), that infection of branches in the autumn took place through leaf scars. Experiments confirming this are described below. A summary of one of these has already been published (Crosse, 1951).

EXPERIMENT I. (1949).

Materials and methods.

Inoculations were made on the three cherry varieties Napoleon, Waterloo and Roundel, as examples of low, intermediate and high disease resistance respectively. The trees were five years old at the time and five trees of each variety were inoculated with strain C2R of *Ps. mors-prunorum*, originally isolated from a diseased spur of Napoleon in early summer 1949. Virulence of this strain was maintained by three consecutive passages, each of approximately 6 weeks' duration, through cherry buds. The " neutral " variety Schrecken was used for this purpose, to avoid the possibility of direct pathogenic adaptation to the experimental varieties. The pathogen was finally re-isolated as strain C2R approximately 2 weeks before the inoculations to leaf scars were made.

Prior to inoculation each tree was marked off into 16 equal groups, each of from 4 to 7 consecutive fruiting spurs, depending on the size of the tree. On each tree the following 8 treatments were then randomized in duplicate, the number of spurs per treatment varying from 40 to 54 :—

Series A. Scars exposed by pulling leaves off forcibly :
(a) Scars inoculated immediately after exposure. A1.

Series B. Abscission stimulated at base of petiole by cutting off leaf laminae 10 days before inoculation :
(a) Scars inoculated immediately and at intervals of 3, 8, 14, 28 and 56 days after exposure. B1–B6 inclusive.
(b) Uninoculated controls. B7.

On 25th October, when the inoculations were begun, abscission was well advanced in spurs of series B and the delaminated petioles readily detached by slight pressure at the distal end. Leaves of series A, not previously delaminated, required some force to detach them.

Bacterial inoculum was prepared by quickly suspending a 48-hours' growth, on nutrient agar plus 2 per cent. glycerine, in 5 ml. sterile distilled water. Inoculations

were made by placing a drop of suspension on the scars, using a loop to avoid damage to the scar tissues. The concentrations of inoculum employed on different dates were not standardized. From 7 to 10 scars were inoculated on each spur, according to its original leaf complement.

It was noted particularly that inoculum applied directly after removal of petioles (treatments A1 and B1) was sucked rapidly into the scar, disappearing completely within a few seconds.

Results.

Development of the disease.

At intervals during the next six months inoculated spurs additional to the above experimental requirements were dissected to follow the progress of the disease.

First visible signs of infection were detected approximately seven weeks after inoculation and at this stage it was still confined to the vascular system and phloem of the leaf traces and secondary wood. A continuous brown discoloration of these elements extended downwards from the leaf scar, often to the waist of the spur. Immediately below the scar the traces were dark stained and waterlogged, but the surrounding parenchyma was still healthy (Fig. 1). The main focus of infection was usually in the waist of the spur, in the main vascular system (Fig. 2). In this region there was already evidence of a limited invasion of the cortex and medulla. By the end of December this had spread to most of the adjacent cortical and medullary tissues, so that an extensive and deep-seated necrosis developed. Meanwhile invasion of the growing apices, from the vascular elements nearer the leaf scar, was proceeding.

By the end of January invasion of the spurs was complete and all tissues, including the buds, were in an advanced state of necrosis. Lenticular pockets, filled with translucent masses of bacteria, were now clearly visible in the phloem and medulla. The spread of infection from the base of dead spurs into branch tissues was first observed at the beginning of February, and by March branch lesions 4–6 cm. long were evident. The margins of these lesions were still diffuse and the clear line of demarcation between diseased and healthy tissues, so characteristic of more mature branch cankers, was not yet evident. Throughout April and May there was a very rapid invasion of the branch tissues, coinciding with renewal of host activity and ascent of the sap. When the progress of infection was halted, towards the end of May, cankers up to 30 cm. long had developed, many of which had girdled the branch and caused die-back. In one tree of Waterloo a branch canker spread to the crotch and stem, with the result that the entire tree was dead by late summer.

The first external signs of infection of spurs on the trees were observed at the end of February, shortly before bud swelling. Diseased spurs were then characterized by the shrunken and relaxed bud scales and by fungal growth on the leaf scars. Small globules of gum, exuding from dead buds and from the axils of spurs and branches, appeared in March and by May many of the diseased spurs were embedded in copious masses of this substance.

The above description applies to treatment B1 and illustrates the rapid onset of disease when conditions are highly favourable. At the other extreme there were spurs in which development was so slow that by May it had progressed little farther than the tissues in the area immediately below the leaf scars. Between these two extremes there was considerable variation, irrespective of treatment or variety. In all treatment/variety combinations, therefore, a range of disease symptoms

finally resulted which were indistinguishable from natural ones previously described (Crosse, 1955). Despite variation, there was clear evidence that disease development was more rapid in Napoleon, and under treatment A1 (Fig. 2), than in the other varieties and treatments. These differences were ultimately reflected in the varying seriousness of the final disease symptoms.

Effects of variety and treatment on disease incidence and disease severity.

In May 1950, incidence and severity of disease were recorded as percentage diseased spurs and severity indices respectively. Severity indices were the mean spur disease ratings calculated by assigning arbitrary ratings of 5, 3 or 1 respectively according as the disease had spread to the branch (spur cankers), was limited to but had killed the spur (dead spur), or had affected part of the spur tissues only (partially diseased spurs). The records are summarized in Table I. The varietal means for disease incidence and severity are based on those treatments (A1 and B1–3) which gave a clear response to the inoculations.

The effect of scar age was obtained by comparing treatments B1–B6. As the time of inoculation and the inoculum were the same for A1 and B1, a comparison of these gave the effect of stimulating abscission prior to inoculation.

a. *Disease incidence.* On all varieties, scars became mainly immune from infection somewhere between 8 and 14 days after exposure, but during this period incidence decreased with increasing scar age. Similar effects for scar age have been described for infection of olive by *Ps. savastanoi* (Hewitt, 1938) and of apple by *Nectria galligena* (Crowdy, 1952).

Disease incidence on the varieties, as shown by the varietal means, was in the same order as their field resistance. Incidence on Roundel relative to Napoleon, however, was higher than is normally encountered under natural infection. There was no evidence that the stimulation of abscission prior to inoculation had any affect on disease incidence.

b. *Disease severity.* Unlike disease incidence, this was not affected by scar age, but was reduced by stimulating abscission prior to inoculation (cf. treatments A1 and B1). The varieties performed, in relation to disease severity, much as they do under natural infection. Thus severity was higher on Napoleon than on Roundel, Waterloo occupying an intermediate position. Branch "die-back", resulting from girdling cankers, ran parallel with disease severity (Table I).

The results of this experiment suggest that disease severity is not necessarily dependent on disease incidence and is mainly characteristic of the variety; a similar conclusion was reached from observations of natural infections (Crosse, 1955).

EXPERIMENT II. (1950).

In this experiment the effect of scar age was examined in more detail. Inoculations were made at shorter intervals and over a smaller period of time. A standard concentration of inoculum, of the same order as that in the first experiment, was used throughout.

Materials and methods.

This experiment was conducted on six-year-old trees of the varieties Napoleon and Roundel, arranged in a plot similar to that used in 1949 but in another locality.

TABLE I.

Disease incidence (percentage spurs diseased) and disease severity (severity indices) on three cherry varieties, following inoculation of leaf scars at varying intervals after removal of the leaves (Experiment I).

	Variety	Interval between exposure of scar and inoculation (days) Treatments in parenthesis							Un-inoculated controls (B7)	Means of treatments A1-B3 inclusive	No. of branches dying back
		0 (A1)	0 (B1)	3 (B2)	8 (B3)	14 (B4)	28 (B5)	56 (B6)			
Disease incidence	Napoleon	98·0	98·0	80·4	35·6	2·2	2·2	2·0	0·0	79·6	
	Waterloo	89·3	85·7	66·7	38·6	7·0	7·5	2·1	6·8	70·7	
	Roundel	90·7	98·1	52·9	31·4	2·2	2·1	1·8	0·0	68·7	
Disease severity*	Napoleon	4·19	4·16	4·81	3·87					4·25	19
	Waterloo	4·14	3·48	3·75	3·59					3·74	7
	Roundel	3·45	3·25	2·41	3·87					3·27	1

* Length of branch cankers was not taken into account in estimating severity indices because of the frequent coalescence of cankers from adjacent spurs. Cankers were, on the whole, longer in A1 than in B1. The difference in disease severity for these two treatments was greater, therefore, than is suggested by the severity indices (see also Fig. 2).

Treatments were randomized as before, with 10 spurs per treatment replicated on 4 trees of each variety. Abscission was induced by delamination of leaves on the selected spurs and the delaminated petioles were removed 10 days later. Inoculations were made immediately and also at 2 or 3-day intervals up to the 12th day after exposure of the scars. The strain of *Ps. mors-prunorum* was W1, isolated from washings from the surface of cherry leaves (var. Waterloo) in September 1950, and passed once through cherry buds (var. Schrecken). The inocula used on different dates were adjusted to the same concentration by reference to a calibration curve showing the relationship between concentration and optical density.

The first inoculations were made on 26th October with a hypodermic syringe the needle of which was blunted to prevent inadvertent wounding of the scar tissues. Rapid suction into the scars, comparable to that in the first experiment, was not observed. As before, extra spurs were inoculated for periodic examination during the following six months.

Results.

Infection followed the same general course as in the first experiment but invasion of the spur tissues proceeded more slowly, the first macroscopically visible signs of infection not being observed until late January. The slower spread of the infection was reflected in the lower severity indices eventually recorded.

Records were made as before and an analysis of variance made of the data. Numbers of diseased spurs were transformed to log. $n+3/8$ before analysis and the means transformed back to numbers of diseased spurs ; for comparison with the first experiment these were converted to percentages. Disease incidence (percentage diseased spurs) and disease severity (severity indices) are shown in Table II. Uninoculated controls, none of which became infected, are not included.

Except for disease incidence and disease severity on Napoleon at 0 days, all other differences were non-significant. Nevertheless, the trend of the results was

Table II.

Effect of age of leaf scar on disease incidence (percentage spurs diseased) and disease severity (severity indices) (Experiment II).

	Variety	Age of scar in days					
		0	2	4	6	9	12
Disease incidence ..	Napoleon	70*	9	4	1	1	1
	Roundel	9	2	2	1	3	1
Disease severity	Napoleon	4·04†	1·70	2·50	0·25	1·25	1·25
	Roundel	1·25	0·50	0·25	1·25	0·33	1·25

Non-significant differences are bracketed.
* $P<0.001$.
† $P<0.05$.

some evidence that incidence decreased as the interval between exposure of the scars and inoculation lengthened, i.e. as the age of the scars increased.

The results of this experiment differed from those of the first in the lower disease incidence and severity obtained over the experiment as a whole and in the large difference in the response of the varieties. Reasons for these differences will be considered later. In the meantime it may be noted that the consistently severe natural infections on the experimental trees in the 4 years preceding the experiment, and the severe disease resulting on Napoleon at 0 days (Table II), precluded the possibility of any special host resistance due to the different locality. The latter result also confirmed the virulence of the bacterial strain used in the experiment.

MECHANISM OF LEAF SCAR INFECTION.

Penetration stage.

From the rapid suction of inoculum into the scars in experiment I and the subsequent localization of early stages of the disease to the vascular system of the fruiting spurs, it was clear that inoculum penetrated through the xylem vessels of the leaf traces. This was confirmed by treating leaf scars with stained bacterial suspensions and with Indian ink. The bacterial suspensions were stained overnight in dilute crystal violet and then centrifuged and washed repeatedly to remove surplus stain. Two hours after treatment, leaf scars and the tissues for a distance of approximately 1 cm. underneath were excised and immediately embedded in ester wax ; on cooling the wax gripped the fresh material firmly and enabled sections to be cut on a rotary microtome without difficulty. Transverse and longitudinal sections of the leaf traces were mounted in water and examined under a 16 mm. objective. Purple masses of bacteria were readily detected in the unstained vessels. Indian ink was diluted 5 times by volume with distilled water before application to the scars and its presence in the vessels was demonstrated under a low power binocular microscope after dissecting away the surrounding cortical and phloem tissues with a sharp scalpel.

In some vessels of the leaf traces no penetration was observed. In others the depth of penetration varied but, alike with bacteria and Indian ink, it rarely exceeded 5 mm. This tendency for suspended particles to accumulate in the upper limits of the vessels was obviously the result of some kind of " sieving out " process, since 1 per cent. acid fuchsin, applied to scars in the same way, penetrated readily and deeply into the main vascular system of the branch.

Acid-fuchsin was also used to examine the effect of scar age on penetration. Dye placed on scars immediately after removal of the leaf could be traced into the vascular system of the branch, where it brought about an intense staining of the vessels. A delay of 6 hours in the application of the dye gave the same result, except that the vascular traces now showed some variation, one from another, in the intensity of staining. After 24 hours, variation in the intensity of staining and depth of penetration, between scars and between leaf traces, was very much more marked, and in 7 out of 30 scars so treated no penetration was observed at all. After 48 hours, visible penetration was observed in only 1 of the 30 treated scars.

Experiments to be described elsewhere have shown that disease incidence

decreases with the number of bacteria, and hence the quantity of inoculum, penetrating the scars. In view of this, the results with acid-fuchsin leave little doubt that the decrease in disease incidence with increasing scar age is related to the reduced amount of inoculum entering the vessels of the leaf traces. On the basis of similar evidence Hewitt (1938) and Crowdy (1952) reached essentially the same conclusion in their studies of leaf scar infection of the olive and the apple respectively.

The same reason probably accounts for the difference in disease incidence between the 1949 and 1950 experiments. As already noted, suction into the scars was more rapid in 1949 than in the following year. By the time evaporation of the infection drops was complete, therefore, it can be assumed that a greater quantity of inoculum had penetrated into scars in 1949 than in 1950.

The higher sap tension apparent in 1949 may have been due to greater limitations on water uptake following a summer of exceptional drought, or to higher transpiratory activity, or to both. If the latter factor was involved, it was not due to environmental conditions, since on the whole these were less favourable for transpiration than in 1950. On the other hand, with the onset of dormancy and leaf senescence, some recession of transpiratory activity probably arises from within the trees themselves independently of environmental conditions. The relative disease levels in the two experiments may, therefore, have been influenced by the fact that leaf fall was later, in relation to time of inoculation, in 1949 than in 1950. This is also a possible explanation of the difference between Napoleon and Roundel in 1950. Both were exposed to the same soil and atmospheric conditions but, as is usually the case, leaf fall was later on Napoleon than on Roundel.

Post-penetration stage.

This was examined in paraffin sections of diseased spurs of the variety Napoleon taken 6 weeks after inoculation. Material was fixed in formalin-acetic-alcohol and embedded by the tertiary-butyl-alcohol technique (Johansen, 1940). Transverse and longitudinal sections, 7–12 μ in thickness, were cut on a rotary microtome and stained in Heidenhain's haematoxylin or carbol thionin (Stoughton, 1930), both counterstained with orange G.

Bacteria were located in the xylem as far down as the base of the spur. In the spiral vessels of the leaf traces, immediately below the scar, they were aggregated in clumps so dense that individual rods could rarely be discerned. The general effect suggested by this distribution was of the choking of the lumina by the sheer number of bacteria entering these comparatively narrow vessels. Lower down, in the larger spring vessels of the secondary wood, the bacteria were more thinly scattered over one or more contiguous vessel segments and individual rods were readily distinguished (Fig. 3). In this region particularly, it is possible that many of the bacteria observed may have originated from the growth of a comparatively few deeply penetrating cells which became lodged in the vessels during their passage downwards from the leaf scars. Although the depth of location of these columns of bacteria was somewhat variable, they tended to be more concentrated in the waist of the spur where, as has already been noted, the invasion of the living cells usually began. No obstructions were observed which would account for the accumulation of bacteria at any particular point in these vessels. In the spring wood the lumina were sometimes completely filled with an amorphous gum-like substance, but this

usually occurred in vessels adjacent to those containing bacteria and was never observed in healthy spurs. It appeared, therefore, to have formed as a response to invasion rather than as an obstruction to penetration. Tyloses were extremely rare in the secondary xylem and never filled the vessels ; no evidence was found that the bacteria had accumulated in front of them.

The failure to find obstructions in the secondary xylem was in marked contrast with the abundance of tyloses which were observed, shortly after leaf fall, in the physiologically redundant vessels of the leaf traces in the region immediately under the leaf scar (Fig. 4).

The explanation of the shallow penetration and irregular distribution of bacteria in the xylem probably lay in the arrangement and structure of individual vessel segments and, in particular, of their cross-walls. The vertical alignment of successive segments was not entirely coaxial, especially as regards the narrower elements which, in tangential section, were frequently arranged in steps. The whole system was further complicated by anastomoses and by some variation in the width of individual vessel segments, the wider ones in particular tending to be expanded at the ends (Fig. 6). The resulting median channels of conduction were thus sinuous.

The vessel segments were 6–20 μ in width and 70–200 μ in length. The wider elements were chisel-ended with oblique cross-walls tending to be orientated in the plane of the radial axis. Each cross-wall was perforated by a pore occupying about half its area, so that a " blind pocket " was formed in the pointed ends of the vessel segments. Smaller vessels were almost tracheidal in character, with long tapered ends which, in contiguous segments, overlapped for considerable distances. In the centre of the overlapping portions the elements were connected by a large pore, indicating their vessel-like nature. In these tapered elements the pore was located in what was virtually a lateral wall, the pointed end thus forming a " cul-de-sac " of considerable depth. The accumulation of bacteria at cross-walls was frequently observed (Fig. 3).

From the xylem the pathogen invaded the living cells, but the manner of the breakout from the vessels has not been ascertained. Bacteria in the large, reticulo-scalariform vessels of the spring wood may have escaped through the pits by active migration following dissolution of the pit membrane. In this case it seemed likely that the physical dimensions of the pit mouth would have constituted a limiting factor. From most vessels containing bacteria there had been no further migration ; whatever the manner of escape, therefore, it did not take place readily and it probably represented a critical stage in the establishment of infection.

In the distal parts of the spur, the phloem and cortical tissues became infected directly from the xylem elements of the leaf trace as these passed obliquely through the cortex (Fig. 5). Lower down, in the secondary xylem, the spring vessels containing the bacteria were separated from the cambium, phloem and cortical tissues by several rows of fibre-tracheids of the autumn wood. In this region the pathogen invaded the phloem, cortex and medulla via the uniseriate and biseriate medullary rays. Early stages of this migration were found where the infection of the medullary ray was still confined to a few cells adjacent to a plexus of vessels containing bacteria (Fig. 7). In later stages the entire ray was infected and in the centripetal and centrifugal regions invasion of the medulla and phloem respectively was proceeding (Fig. 8). The pathogen reached its maximum development in the medullary rays of the phloem area, where the ray cells were disintegrated and replaced by lenticular

pockets of bacteria, with the long axis of the pockets arranged in the vertical plane. Although there was no evidence that the phloem elements between the rays had been invaded, these were, nevertheless, blackened and apparently devoid of cell contents.

At the advancing edges of the infection in the medullary rays, bacteria were present only between the cells. Intra-cellular migration was observed in the cortical parenchyma in the more distal regions of the fruiting spur. At a later stage large cavities, filled with bacteria, developed in the cortex and rays ; this was accompanied by the disappearance of cell material, including the cell walls, and by the formation of lysigenous pockets.

In Experiment 1 the number of vessels containing bacteria, and the number of infected medullary rays, was very much greater under treatment A1 than under treatment B1. This was probably the reason for the more rapid spread of the disease in spurs of treatment A1. Comparison between treatments A1 and B1 suggests that the rate of development and the final severity of disease is determined by the number of vessels penetrated by the pathogen and that the effect of the abscission processes is to reduce the number of vessels available for entry. This reduction may be related to tylose formation and merits further investigation.

Effect of the pathogen on the host tissues.

Certain features of the invasion, e.g. intercellular penetration, killing in advance of penetration and dissolution of cell material, followed a pattern familiar in many other bacterial plant diseases (Hill, 1930 ; Skoric, 1927 ; Miller, 1929 ; Zaumeyer, 1932) and were of interest because they suggested possible mechanisms involved in pathogenesis.

Intercellular penetration.

As in plum (Erikson, 1945), the initial colonization of cherry tissues by *Ps. mors-prunorum* was intercellular. Considerable bacterial growth was observed in the intercellular spaces of the cortex without much apparent damage to adjacent cells, even when these were completely separated from their neighbours by bacterial masses (Fig. 9). In the medullary rays, where intercellular spaces are absent, cells were peeled apart during the progress of bacterial growth (Fig. 10). This presumably followed dissolution of the middle lamellae and suggested the activity of pectin-degrading enzymes which Mills (1949, 1950) has shown may be produced extra-cellularly by young *in vitro* cultures of *Ps. mors-prunorum* and of the related cherry pathogen *Ps. syringae* (syn. *Ps. prunicola*).

Killing in advance of penetration.

The killing of living cells in the absence of penetration by the pathogen was a consistent and conspicuous feature, particularly in the phloem and xylem (Fig. 8), and suggested the activity of a diffusible microbial toxin. Erikson (1945) noticed a similar killing of plum tissues and also believed it to be toxigenically induced. Erikson and Montgomery (1945) were able to reproduce the necrosis by injecting into plum tissues cell-free filtrates of cultures of *Ps. mors-prunorum* or extracts of the bacterial cells. Because active filtrates were obtained only from aged cultures and the most active ones from the cell extracts, they concluded that the lethal principle

PLATE I

Fig. 1.

Diseased spur (left), seven weeks after inoculation. The infection is still confined to the leaf traces. Compare healthy spur (right). (×2.)

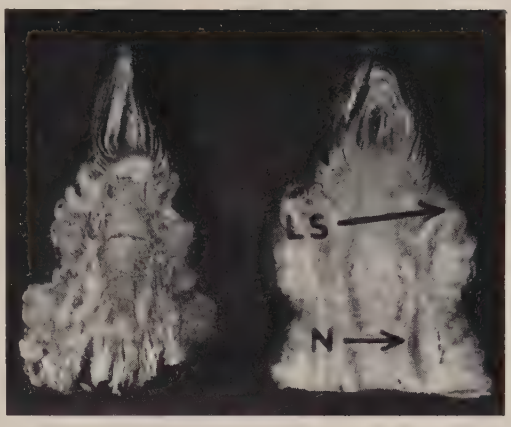

Fig. 2.

Median sections of diseased spurs, eight weeks after inoculation. In treatment A1 (left) the entire spur, including the bud, is now dead and small pockets, originally filled with bacteria, are present in the phloem, cortex and medulla. In treatment B1 (right) the infection is still mainly confined to the vascular system and phloem and is centred in the waist of the spur (N), some distance below the leaf scar (L.S.). (×2.)

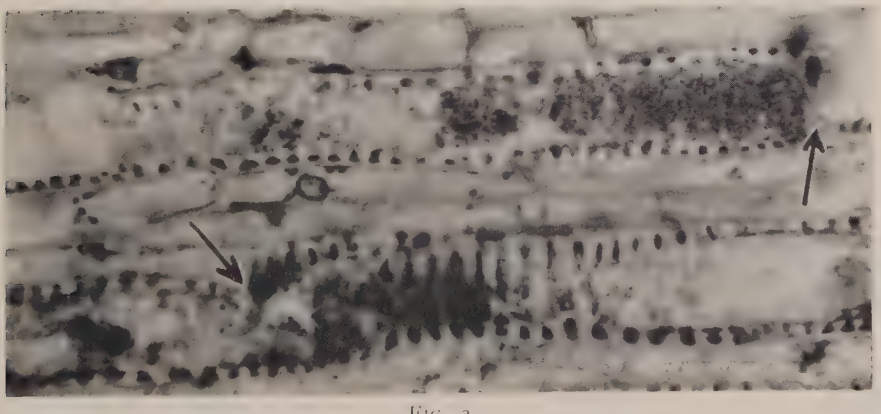

Fig. 3.

R.l.s. diseased spur in early stages of leaf scar infection, showing bacteria in the reticulately thickened spring vessels of the secondary wood. At this particular point there has not yet been any migration into the adjacent wood parenchyma. Note cross walls indicated by arrows. (×980.)

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FIG. 4.

R.l.s. of a leaf scar showing tyloses in the spirally thickened vessels of the leaf trace, immediately under the face of the scar, at the time of inoculation in October, 1949. Abscission had been stimulated by delamination 10 days beforehand. (×400.)

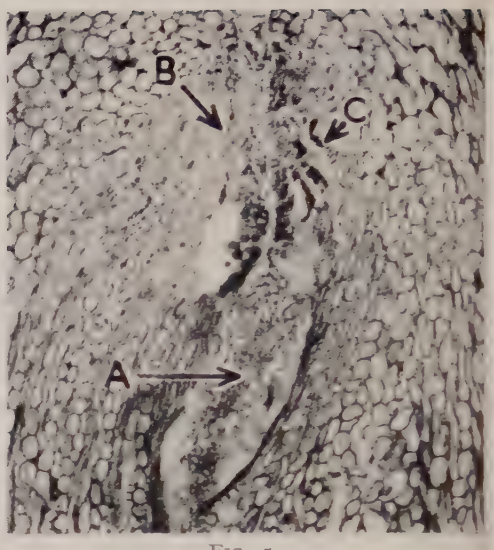

FIG. 5.

T.l.s. of a diseased spur showing lysigenous cavity (A) developing in the cortical tissues at the distal end of the spur. The infection originated from the diseased leaf trace (B), certain vessels of which can be seen filled with bacteria (C). (×115.)

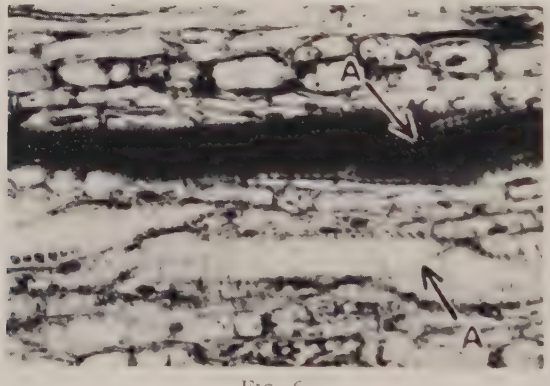

FIG. 6.

T.l.s. of secondary wood of a diseased spur. The upper of the two vessels is filled with darkly staining masses of bacteria. Note oblique perforated cross walls (A) and tapered and slightly expanded ends of the vessel segments. (×400.)

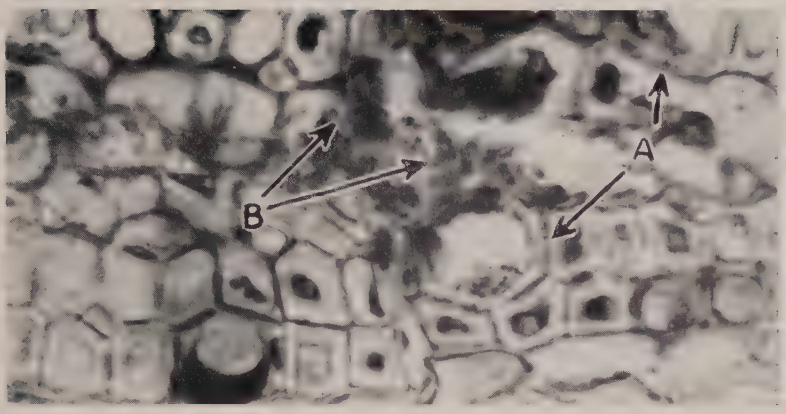

FIG. 7.

T.s. of the secondary wood of a diseased spur. Note vessels containing bacteria (A) and an early stage in the migration of the bacteria from the vessels to the cells of the medullary ray (B). (×870.)

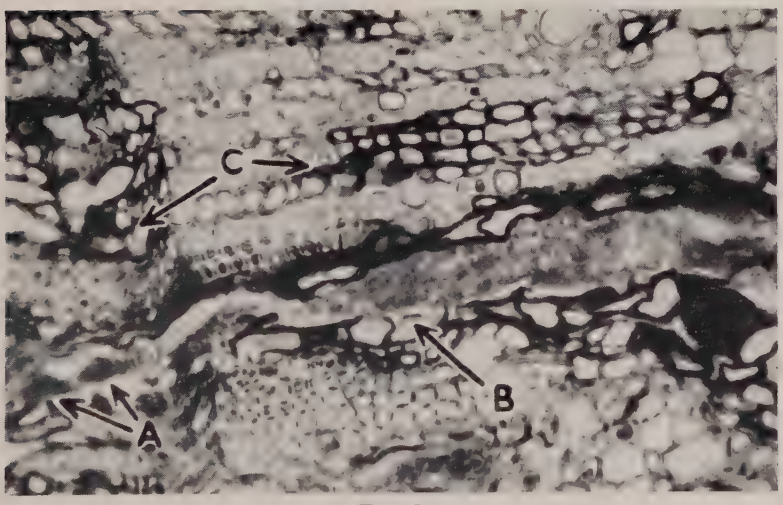

FIG. 8.

T.s. of diseased spur showing bacteria in a medullary ray of the secondary wood. At A the bacteria have crossed the cambium into the phloem. The beginning of intracellular penetration can be seen at B. Dead cells (C), with blackened walls and disorganized cell contents, were frequently observed at some distance from the areas actually colonized by the pathogen. (×400.)

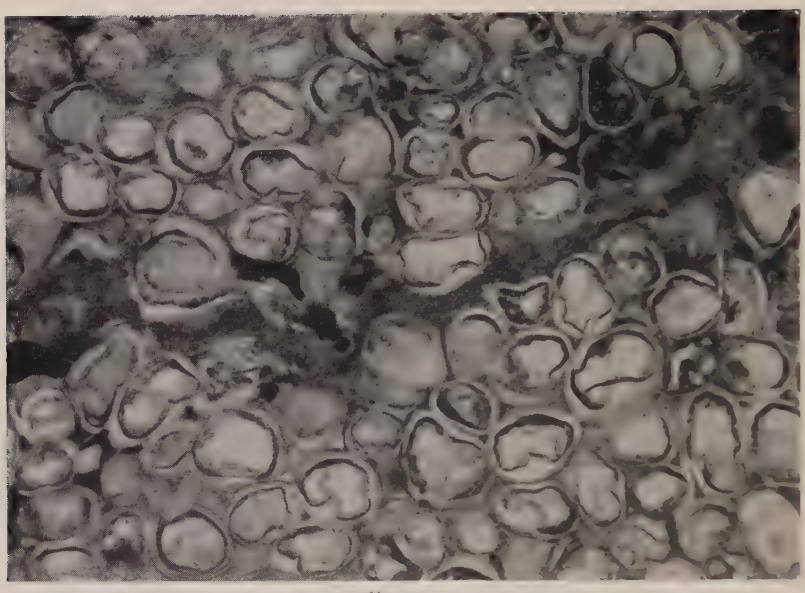

FIG. 9.

R.l.s. of diseased spur showing intercellular invasion of the cortical tissues. In some cells the protoplasts were shrunk, but apart from this the tissues appeared undamaged. (×440.)

FIG. 10.

R.l.s. of infected medullary ray showing intercellular penetration by bacteria. (×1125.)

was an *endotoxin* liberated on the autolysis of bacterial bodies. There was evidence that the toxin was a protein and that it was more damaging to the tissues of a plum variety of low than to one of high disease resistance. Toxic filtrates were also obtained from other bacteria pathogenic to plum but not from non-pathogenic species or saprophytes. There was indirect evidence, therefore, that a toxin might be produced *in vivo* and that it played some part in pathogenesis. Erikson and Montgomery (1945) were in fact of the opinion that it was responsible for the progression of cankers caused by *Ps. mors-prunorum* in plum trees, possibly in conjunction with metabolites produced by the host tissues.

The evidence from cherry is not incompatible with the idea of an endotoxin of this nature. Thus necrosis, as shown by the blackening of the cells walls and disappearance or disorganization of the cell contents, was invariably most widespread near areas where bacteria had been present or actively growing for some time and where therefore some autolysis may have occurred. By contrast, apart from the plasmolysis of occasional cells, there was little damage to the host tissues during the early stages of intercellular colonization when bacterial autolysis was less likely. On the other hand, this same difference could equally well result from an extracellular secretion if this reached lethal concentrations only after considerable bacterial growth had occurred.

In regions of extensive bacterial growth, where the supply of immediately available food materials might be expected to be limiting, it is possible to perceive special advantages of a toxin mechanism. By killing tissues in advance of penetration, it would permit the diffusion of soluble cell constituents from these tissues to the areas actually colonized by the pathogen. From the phloem, particularly, organic nutrients would be available in abundance, and this could explain why maximum bacterial growth always occurred in the medullary rays of that tissue.

Dissolution of cell material.

Lysigenous cavities were occasionally observed in the cortex without any distinct necrosis of the cells marking the periphery of the cavity. The contrast between this and the extensive necrosis in the phloem and xylem without any cell dissolution suggested that the factor concerned in necrosis was not responsible for cell lysis. It is possible that a cellulase system may be involved in the disappearance of cell wall material. According to Burkholder (1948), however, the possession of such a system by any plant pathogenic bacterium has not yet been demonstrated.

DISCUSSION.

These investigations confirm that *Ps. mors-prunorum* invades cherry trees in the autumn through leaf scars. During rain, inoculum is washed on to scars and sucked into the vessels of the leaf traces. In periods of prolonged rain this process may be continuous, and considerable numbers of bacteria may thus accumulate in the vessels, even from dilute inoculum. Wind-driven rain appears to provide the ideal conditions for infection, by the simultaneous removal of leaves and distribution of inoculum. It is clear from the inoculation experiments, however, that removal of the leaf and arrival of the infection drop need not necessarily coincide and that a high level of infection might occur in normal rain which followed some time after a dry windy day.

The pathogen penetrates into the vascular system, from which it invades the living tissues of the spur and ultimately those of the branch. There is no evidence

of direct penetration into the vessels of the branch. The protective layer of the scar formed by the ligno-suberization of peripheral parenchyma during or soon after leaf abscission, while precluding invasion of the scar by progressive colonization of the living tissues exposed at leaf fall, does not prevent infection, against which tyloses and, possibly, gum formation in the vessels appear to be the only possible defensive mechanisms.

As an avenue of infection, the leaf scar has an advantage in that, after entrance of the pathogen into the xylem vessels of the trace, the early and critical stages of infection, i.e. invasion of the living tissues, is completed in a relatively protected environment. At the same time it imposes certain limitations on a pathogenic micro-organism, which may explain the rarity of this type of infection. In the first place it is likely to be of importance only where there is some degree of continuity of the aerial parts, as in woody perennials. Secondly the infective particle must be smaller than the diameter of the vessels. In this respect Brookes and Moore (1923) noted that the spores of certain fungal wood parasites, e.g. *Stereum purpureum*, are universally small. Thirdly, for obvious reasons, penetration is most likely to be effected by organisms which are normally distributed in rain, e.g. *Nectria galligena* (Munson, 1939) and most phytopathogenic bacteria. Finally, in deciduous hosts, the pathogen must be capable of actively invading the tissues during the dormant period of the host, as are in fact *N. galligena* and *Ps. mors-prunorum*.

On account of their small size and their distribution by meteoric water, the bacteria are obviously well adapted to leaf scar infection and it is perhaps significant that two of the three cases of this type of infection which, so far as is known, have now been demonstrated, should be caused by these organisms.

SUMMARY.

Autumnal inoculations of leaf scars of cherry fruiting spurs with *Pseudomonas mors-prunorum* resulted, by the following May, in disease symptoms exactly comparable to those occurring naturally. Cherry varieties differing in their resistance to the disease responded to inoculation in much the same way as under conditions of natural infection. These results confirmed previous field evidence of leaf scar infection.

The susceptibility of scars to infection decreased as the interval between removal of the leaves and inoculation was lengthened, and there was evidence that this was related to the quantity of inoculum penetrating the scars. Difference in the amount of disease resulting from two experiments carried out in successive years was attributed to similar reasons.

It was shown that inoculum entered scars via the xylem vessels of the leaf trace and frequently penetrated into the main vascular system of the fruiting spur. From here bacteria invaded the medullary rays and then spread along these into the cortex and pith. Maximum bacterial growth occurred in the medullary rays of the phloem, but there was no evidence that the latter tissue was invaded. The early stages of colonization were intercellular, but in later stages intra-cellular penetration and the dissolution of cell material were observed. Evidence of the killing of cells in advance of penetration was obtained and this, together with other histological effects, is discussed in relation to an existing theory of endotoxin production by the pathogen.

The advantages and limitations of the leaf scar as an avenue of infection are considered briefly in relation to the nature of the pathogen.

ACKNOWLEDGMENTS.

I wish to thank Dr. R. V. Harris for his continued interest in these investigations, Mr. G. H. Freeman for the statistical analysis and Mr. E. Yoxall Jones for the photographs. I am also indebted to Miss M. Bennett and her recording staff for help in the field experiments.

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BOOK REVIEWS

BOTANY. PRINCIPLES AND PROBLEMS. By EDMUND W. SINNOTT and KATHERINE S. WILSON. 5th Edition. (London : McGraw-Hill Publishing Company Ltd. 1955. Pp. 528, illus. Price 48s. 6d.)

This new edition of a standard work, first published thirty-two years ago, is another of those American text books that make one wonder whether the students of universities in the United States must not be much better off than their opposite numbers in the United Kingdom. It is beautifully produced and illustrated, yet, at its price, seems more likely to be found in libraries rather than on the shelves of undergraduates' bed-sitting rooms.

Yet, it is a fine book that must be invaluable to embryo botanists ; indeed its five editions testify to its usefulness to several generations of students, who must have enjoyed discussing the thought-provoking questions that conclude each chapter.

The present edition is substantially revised and brought up to date, with many new illustrations added. I like especially the appendix giving the Greek and Latin roots commonly used in the construction of technical botanical terms. In these days when, as a rule, Latin and Greek receive scant attention in our schools, the young botanist must find this list a valuable aid to his understanding ; and dare one hope that those who coin new terms for the future will also use the list, bearing in mind the authors' final injunction " Avoid combining a Greek with a Latin root " ?

It seems a pity that this text book could not have been produced with half the number of illustrations, with a less extravagant format and at half the price, when so many more of those for whom it is intended might possess it for themselves.

T.N.H.

PRACTICAL HORTICULTURE. By JAMES S. SHOEMAKER and BENJAMIN J. E. TESKEY. (New York : John Wiley & Sons Inc. ; London : Chapman & Hall Ltd. 1955. Pp. 374, illus. Price 34s.)

The authors of this book are members of the staff of the Department of Horticulture at the Ontario Agricultural College, Guelph, and it is written primarily for students of vocational horticulture and for gardeners, presumably in Canada. It has little in it, at least as far as fruit is concerned, that is of application in England and, even in North America, its value would appear to be limited to those who intend to practice horticulture in Southern Ontario.

Within its 370 pages are compressed the whole range of horticultural crops, flowers, ornamental plants, vegetables and fruit. It is hardly surprising that much of the information given is superficial.

Admittedly, I can judge from experience only that part of the book which deals with fruit. These chapters contain much general information but there is little that is specific. A few varieties are listed but not described. The information given about fertilizers and the control of pests and diseases is very sketchy. Perhaps it is because there has not been a really hard winter in the fruit growing parts of Canada for twenty years that winter injury to fruit trees is not mentioned.

The book is well produced and liberally illustrated. It is hoped it will be of value to those for whom it is intended. T.N.H.

SOIL FERTILITY. By C. E. Millar. (London: Chapman & Hall Ltd. 1955. Pp. xi+436, illus. Price £2 14s.)

This new book, written by the late Professor Millar of Michigan State College, deals with the fundamental principles of soil fertility in relation to plant growth, with the major emphasis on the plant itself. Its nineteen chapters cover a very wide field indeed, including such aspects as: the constitution of soils, the total amount and distribution of plant nutrients in American soils, methods for determining the availability of both major and trace elements, the chemical composition and use of organic and inorganic fertilizers, soil management and field experimentation. Although the examples are taken almost entirely from American work the deductions made from them are of universal application. The author has deliberately avoided too much applied material, but the book contains an appreciable amount of useful reference data, and each chapter ends with an adequate bibliography.

It is essentially a textbook for students and as such can be recommended because of its lucid style and the manner in which the information is presented. C.B.

THE GLADIOLUS FOR GARDEN AND EXHIBITION. By D. Gourlay Thomas. (London: W. H. & L. Collingridge Ltd. 1955. Pp. 96, illus. Price 15s.)

The Rev. Gourlay Thomas's latest gardening book is written in a light and easy style which makes it as suitable for fireside armchair reading as for serious study. In a racy and humorous manner, combined with boundless enthusiasm, the author makes his reader feel that he must grow gladioli at all costs and gives him ample assistance in doing so. Valuable hints on growing, exhibiting and storing gladioli are enlivened by witty observations and apt illustrations. Pests and diseases are dealt with in the same lively style and the reader is not left with the impression that all his plants must inevitably succumb to some dire complaint unless he takes the most elaborate precautions. One chapter describes the raising of new varieties and the mechanics of cross pollination are clearly explained, although it seems pointless to mention the use of "hormones and drugs such as colchicine and its derivatives" unless the subject is to be developed in greater detail.

A number of good photographs are included in the book to illustrate various types of gladioli such as Large Flowered, Miniature, Primulinus and Butterfly. The author's personal recommendations of modern varieties of Large Flowered and Primulinus types are extremely useful, as one is apt to be bewildered by the great number which are listed in catalogues. The Rev. Gourlay Thomas states in his foreword that he does not intend to deal fully with the history of the plant, and in addition he makes only brief reference to the Nanus and Herald types; such omissions make the book of less value to the serious student. As long as the prospective reader realizes that only the Primulinus, Miniature and Large Flowered gladioli are fully dealt with and does not expect the book to cover the complete range, I recommend it as a useful and readable book offering much sound information on this delightful plant. M.P.

SOIL ZOOLOGY. Edited by D. K. McE. Kevan. (London : Butterworths Scientific Publications. 1955. Pp. 512, illus. Price 55s.)

This work is not, and does not claim to be, a complete textbook of soil zoology. It is an edited collection of the papers read and techniques demonstrated at a symposium and colloquium organized by the University of Nottingham School of Agriculture on the occasion of their second (1955) Easter School in Agricultural Science. The subject of soil zoology was chosen because it was felt that the importance of the immensely varied and numerous animal populations in the soil was not fully appreciated by workers in other aspects of soil science. Although this situation may be largely because soil scientists and field officers are not usually trained in zoology, another important factor is the scattered nature, both in subject matter and in space, of the zoological work which has so far been done in this field. The organizers, in attracting such a comprehensive gathering of specialists, did much to improve this position, and thereby provided members of the course with an opportunity to survey and select from an unusually wide zoological field. Fortunately the matter has now been taken a step further and this store of knowledge has been opened to a much larger public by the prompt presentation of this concise and readable account of the proceedings.

The bulk of the contributors are British and nearly all are European. The subject is introduced by papers from Professor Kühnelt of Vienna, and by a helpful contribution from the Editor, who was also Organizing Secretary of the Symposium, on the identification of soil and litter animals. The latter paper, which covers the full range from protozoa to vertebrates, is supported by an illustrated key to the orders and sub-orders. Overseas contributors include Professor Tischler of Kiel, Professor Kubiena of Madrid and Dr. Overgaard Nielsen from Denmark, while British speakers come mainly from Rothamsted Experimental Station, university staffs, the National Agricultural Advisory Service, the Nature Conservancy, and the British Museum (Nat. Hist.). The contents are divided into two main parts. The first is of general scope and covers pedology, ecology, applied aspects and zoophagous fungi, including several papers by specialists on groups such as nematodes, annelid worms and various arthropods. The second part is concerned with methods, many of which were the subject of practical demonstrations at the symposium. Sampling and estimation techniques for arthropods, annelid worms and nematodes occupy about two-thirds of this part, and are followed by sections on culture methods (for protozoa and arthropods), preparations (of animals and soil sections) and techniques for physiological studies. Four separate indices conclude the book, one of authors, one of animal genera, one of plant genera and lastly a comprehensive general index.

Many of the papers are illustrated with line drawings or plates and include details of the discussions which followed their presentation to the symposium. Few if any, cannot be readily understood by anyone with a modicum of biological knowledge or, indeed, of plain commonsense. Thus, while this book offers little in his own subject to attract the specialist, there is much to broaden his view of the work of others, an exercise which cannot be other than helpful in a study connected with so complex an environment as the soil. There is also much to attract the beginner in any one of a variety of fields and there is little doubt that this compilation will play a real part in stimulating progress in many aspects of this extensive and difficult field of work.

R.S.P.

CHEMISTRY OF THE SOIL. Edited by FIRMAN E. BEAR. (New York : Reinhold Publishing Corporation. 1955. Pp. 373, illus. Price 70s.)

It would require a reviewer as qualified as Professor Firman E. Bear, the editor of the monograph, to do justice to this work, which is to be highly commended.

The volume is divided into ten chapters and an appendix, dealing in sequence with the following aspects of soil chemistry : soil development, chemical composition and colloid chemistry of soils, cation and anion exchange, organic matter, nutrient fixation and oxidation-reduction processes in soils, soil reaction, trace element and plant nutrition, while the appendix very adequately covers methods in soil analysis.

The authors of these chapters, each an indisputable authority on the aspect of soil chemistry he covers, deal critically and comprehensively with their subjects, and collectively have presented a full review of present knowledge on the chemistry of the soil.

Further excellent features of this monograph are the many sets of useful data, the critically selected lists of references contained in the bibliographies at the end of each chapter, and the index.

This volume is of unquestionable value to soil scientists and there can be few engaged in agricultural or horticultural research who will not find one or more of its chapters useful and stimulating. J.E.G.

SALAD AND OTHER FOOD CROPS IN GLASSHOUSES. Ministry of Agriculture, Fisheries and Food Bulletin No. 143. (London : H.M. Stationery Office. 1955. Pp. 51, illus. Price 2s. 6d.)

The bulletins of the Ministry of Agriculture are essential to all those engaged in commercial horticulture and are, in addition, exceedingly useful for students and those already engaged in research on the various crops concerned. We have now received a new edition of this bulletin, prepared by the Glasshouse Group of the National Agricultural Advisory Service, and we may say without more ado that, emanating from such a source, we can be sure that, as with its companion publications, it contains up-to-date and authoritative advice on the various crops covered. Further, this advice is stated concisely, in a readable form and at a price within the means of everyone.

This bulletin contains chapters on beans, cucumber, lettuce, melon, mint, mustard and cress, and a variety of other crops ranging from egg plants to strawberries. It also includes some information on the control of pests and diseases, with references where necessary, and a useful appendix giving details of the John Innes seed and potting composts. T.N.H.

A GLOSSARY OF TERMS USED IN PRUNING FRUIT TREES. Reprinted from Scientific Horticulture, Volume XI, 1952-54. (The Horticultural Education Association, c/o Gibbs & Sons, Canterbury, Kent. 1955. Pp. 8, illus. Price 1s. 3d.)

This glossary, prepared by the Association's Fruit Committee, is designed to clear up confusion in the many terms used in pruning pome and stone fruits in commercial fruit plantations. It is now available in reprint form and all those either teaching or learning the art of pruning, who do not already possess a copy, are strongly advised to acquire one before the next pruning season.

NOTICE TO CONTRIBUTORS

1. The **Journal of Horticultural Science** is an official organ of the Horticultural and Agricultural Research Station, Long Ashton, Bristol, and the Horticultural Research Station, East Malling, Kent.

The Publication Committee welcome horticultural papers from all other sources.

2. All **manuscripts** should be in double-spaced typewriting, with a good margin, and should be addressed to the Editors, East Malling Research Station, near Maidstone, Kent, England. Papers must be written concisely. Historical reviews should be as short as possible and only such references to literature as bear directly on the subject should be included. Tables and illustrations, if any, must be only such as are absolutely essential for clear presentation.

3. Authors of papers containing extensive **numerical observations** should consult the recommendations of the British Association Committee on Biological Measurements, 1927, obtainable from Burlington House, London, W.1. Price 6d. Arrangements have been made whereby original data too extensive for complete publication may be deposited at the British Museum (Natural History), London, S.W.7, or with the Royal Society of Edinburgh, 22 George Street, and thus be available for consultation by other workers. Full bibliographical reference to the publication in which the results are summarized must be supplied with the data.

4. Each paper must conclude with a concise and critically correct **summary** of its actual content. It should contain any new or verified constants as well as any other critical data of permanent value but not tables or graphs. Any organism mentioned should be given its scientific name.

5. Where a paper is of a highly technical nature it should be preceded by a brief **synopsis**, in which its contents are simply and clearly stated for the benefit of non-technical readers.

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